

# "Scientific Opportunities for a Global Algal Revolution"

# **Program and Book of Abstracts**

Edited by Solène Connan, Emeline Creis, Bertrand Jacquemin, Gabriel Markov and Philippe Potin





### EPC8 is Supported by



# Content

EPC8 programme	4
List of Posters	26
Book of Abstracts	39
Index of Authors	352

### **Brief Statement Organizers**

When we commenced organising EPC8 we still faced the real possibility that the pandemic could throw the organisation off the rails. Nonetheless, we decided against 'hybrid' because four years after EPC7-Zagreb it would be great to finally meet face-to-face again. E-meetings save costs and time and produce less CO<sub>2</sub>, but who is not tired of meeting on a computer screen?

We organised the program with 20 symposia in six overarching themes, covering all that keeps us busy. All symposia accommodate micro- and macroalgal presentations to inspire you with research adventures including those in 'the other phycological denomination.' Since all of you teach, reach out, and publish, we included symposia about those topics as well. Each conference day has four parallel symposia, from different themes, so as to minimise your choice-stress. Since, posters are integral to the information exchange, we put the poster sessions centre-stage and have devoted plenty of time to them.

We went out of our way to ensure gender balance and diversity. We invited symposium conveners from all over Europe and beyond, and we asked them to consider especially 'young, rising stars' as keynote speakers. Young scientists are our investment in the future of phycology.

Enjoy EPC8 and Brittany!

Inka Bartsch, Solène Connan, Philippe Potin & Wiebe Kooistra

### The local Committee of EPC8:

Having less  $CO_2$  productive conference was still among our preoccupation, and this is the reason why we decided to limit the number of goodies and publish only a digital version of the abstract book, still in cooperation with European Journal of Phycology.

Chairs of the committee: Solène Connan & Philippe Potin

Members of the committee:

Azzedine Badis | Yacine Badis | Eva Bucciarelli | Thomas Burel | Jonas Collen | Mark Cock | Emeline Creis | Eric Deslandes | Christophe Destombe | Simon Dittami | Laure Guillou | Bertrand Jacquemin | Cécile Klein | Martial Laurans | Johann Lavaud | Catherine Leblanc | Marc Long | Gabriel Markov | Sylvain Petek | Ian Probert | Andrés Ritter | Philippe Soudant | Nathalie Simon | Valérie Stiger-Pouvreau | Jill Sutton | Myriam Valéro

Organisers:



## **EPC8** Programme

The programme includes the names of the presenting authors only. As a professional courtesy, you are kindly requested not to take photographs in the lecture rooms. If you are interested in data presented, please contact the presenter directly. We also would like to remind everybody to switch off their mobile devices during the presentations. In bold and \*, talks in the oral competition.

Sunday 20<sup>th</sup> August

3:00pm - 7:00pm	Registration	Arena Hall
6:00pm – 9:00pm	Ice breaker reception	Arena Hall
Monday 21 <sup>st</sup> Au	gust	
8:00am – 6:00pm	Registration	Arena Hall
8:30am – 9:00am	Opening Ceremony	Room "Tali ebrel"
9:00am - 10:00am	Plenary Lecture	Room "Tali ebrel"
Sexual and asexual r	CHRISTOPHE DESTOMBE eproduction in rhodophytes: evolution, genetic divers	ity and adaptive strategy
10:00am - 11:00am	Symposia 01, 04, 13 and 15	
Symposium 01		Room "Tali ebrel"
	<b>Taxonomy and Systematics</b> Convenors: Fabio Rindi & Patrick Kociolek	
10:00am – 10:30am	PATRICK KOCIOLEK - One step forward and two s taxonomy, systematics and classification of diatoms	-
10:30am – 10:45am	DOVILE BARCYTE - The resurrected genus Hypogenetic, morphological, and genomic divers green algal order Chaetopeltidales	· ·
10:45am – 11:00am	JAN POKORNÝ - New insights into the phyloger Merismopedia and Microcrocis (Cyanobacteria) ger	-
Symposium 04		Room "Tali moan"
	Algae and their intimate partners Convenors: Johan Decelle & Aschwin H. Engelen	
10:00am – 10:30am	SUHELEN EGAN - Factors that influence microbiome in seaweeds	e development and disease
10:30am - 10:45am	*CORALIE ROUSSEAU - The <i>Ascophyllum nodosi</i> of microbial communities over one-year in situ s	÷
10:45am – 11:00am	*LUNA VAN DER LOOS - The interplay of acclimation to salinity	<i>Ulva</i> and its bacteria:

Symposium 13 Al	Room "Tali friz" gae and ecosystem functioning: the genomics perspective Convenors: Flora J. Vincent & Bente Edvardsen	
10:00am – 10:30am	CHANA KRANZLER - Viral infection dynamics in marine diatoms across environmental and ecophysiological gradients	
10:30am – 10:45am	VALERIA DI DATO - Prostaglandins in marine diatoms	
10:45am – 11:00am	LENKA ŠTENCLOVÁ - Iron monopolization versus community service: the two faces of cyanobacterial beta-hydroxy aspartate lipopeptides	
Symposium 15	Room "Tali melen"	
	Algae as producers of valuable compounds Convenors: Abdelfatah Abomohra & Alexei Solovchenko	
10:00am - 10:30am	DIETER HANELT - Recent advances in sustainable applications of Seaweeds	
10:30am – 10:45am	SIMON OIRY - Precision aquaculture drone mapping of the spatial distribution of <i>Kappaphycus alvarezii</i> biomass and carrageenan	
10:45am – 11:00am	HUGO PLIEGO-CORTÉS - Effect of the Instant Controlled Pressure Drop (DIC) Technology for extraction of seaweed compounds	
11:00am – 11:25am	Coffee Break Arena Hall + Room "Pioka"	
11:30am – 12:30am	Symposia 01, 04, 13 and 15	
Symposium 01 (cont	inued) Room "Tali ebrel" Taxonomy and Systematics Convenors: Fabio Rindi & Patrick Kociolek	
11:30am – 11:45am	MAURYCY DAROCH - The importance of genomics in polyphasic identification of novel thermophilic cyanobacteria	
11:45am – 12:00am	*SVATOPLUK SKOUPÝ - 200 years of Microcoleus - untangling the taxonomy in species continuum	
12:00am – 12:15am	*MARGOT ARNOULD-PÉTRÉ - Is structural colour in the red algal genus Chondria (Rhodomelaceae, Chondriae) taxonomically significant?	
12:15am – 12:30am	*DANIEL ÁLVAREZ-CANALI - Assessing the molecular diversity of <i>Sargassum</i> (Phaeophyceae) in the NE Atlantic Ocean	
Symposium 04 (cont	Symposium 04 (continued) Room "Tali moan"	
	Algae and their intimate partners Convenors: Johan Decelle & Aschwin H. Engelen	
11:30am – 11:45am	LEILA TIRICHINE - Combined <i>in vivo</i> and <i>in situ</i> genome-resolved metagenomics reveals novel symbiotic nitrogen fixing interactions between non-cyanobacterial diazotrophs and microalgae	
11:45am – 12:00am	MARIE WALDE - The impact of biotic interactions on subcellular structure - an automated 3D imaging approach for marine diatoms	

12:00am – 12:15am	YESEREN KAYACAN - Assembly of diatom-associated bacterial communities under phosphate stress	
12:15am – 12:30am	SAUVANN PAULINO - Temporal dynamic of <i>Ulva</i> sp. epiphytic bacteria and associated surface metabolome	
Symposium 13 (continued)       Room "Tali friz"         Algae and ecosystem functioning: the genomics perspective       Convenors: Flora J. Vincent & Bente Edvardsen		
11:30am – 12:00am	SIMON DITTAMI - Exploring and manipulating the Saccharina latissima microbiome	
12:00am – 12:15am	YUE WU - Exploring the Role of microbiome and epigenetics in shaping the response of <i>Phaeodactylum tricornutum</i> to hypersalinity	
12:15am – 12:30am	ANUSUYA WILLIS - A global genomics study of <i>Raphidiopsis raciborskii</i> , demonstrating the power of culture collection collaborations	
Symposium 15 (cont	inued) Room "Tali melen"	
	Algae as producers of valuable compounds Convenors: Abdelfatah Abomohra & Alexei Solovchenko	
11:30am – 11:45am	HUGO MEAR - Extraction of soluble proteins by bead milling from <i>Tetraselmis chui</i> in two different physiological states	
11:45am – 12:00am	DANIEL REMIAS - Zygnematophyceae and their complex phenolic compounds - a biochemical strategy preceding the flavonoids of land plants?	
12:00am – 12:15am	LAURA RIEUSSET - Purification of mycosporin-like amino acids by centrifugal liquid chromatography for improved structural and biological characterisation	
12:15am – 12:30am	ALLA SILKINA - Application of algal technology to convert waste nutrients produced from the anaerobic digestion of farm waste and brewery process to produce biomass for feed application	
12:30am – 1:25pm	Lunch Room "Pioka"	
1:30pm - 3:00pm	Symposia 01, 04, 11 and 15	
Symposium 01 (cont		
	<b>Taxonomy and Systematics</b> Convenors: Fabio Rindi & Patrick Kociolek	
1:30pm – 2:00pm	FREDERIK LELIAERT - The future challenges of seaweed systematics	
2:00pm – 2:15pm	CRAIG SCHNEIDER - On the application of European binomials for western Atlantic marine macroalgae by 19th century taxonomists	
2:15pm – 2:30pm	PHAIK-EEM LIM - Proposal for a global taxonomic and nomenclatural framework for eucheumatoids (Rhodophyta)	
2:30pm – 2:45pm	VIVIANA PEÑA - Diversity of coralline red algae from ecologically relevant habitats in Corsica, western Mediterranean	

2:45pm – 3:00pm M. BEATRIZ BARROS-BARRETO - Phylogenetic analysis of the red algal tribe Ceramieae reveals multiple morphological homoplasies but defines new genera

Symposium 04 (cont	
	Algae and their intimate partners Convenors: Johan Decelle & Aschwin H. Engelen
1:30pm – 1:45pm	ERIK JAN MALTA - Dynamics of the <i>Ulva ohnoi</i> (Chlorophyta, Ulvales) microbiome under different cultivation conditions
1:45pm - 2:00pm	ANANYA KEDIGE RAO - Integration of stolen chloroplasts in marine plankton
2:00pm – 2:15pm	NADEESHANI DEHEL GAMAGE - Could algae - bacteria partnership accelerate growth and lipid production of oleaginous benthic diatoms?
2:15pm – 2:30pm	ZUJAILA NOHEMY QUI MINET - How does light affect the presence and antibacterial activity of <i>Phaeobacter</i> in the <i>Ulva ohnoi</i> holobiont?
2:30pm – 2:45pm	LILIANA MUÑOZ - Natural biocontrol of a <i>Porphyra</i> sp. epiphyte on farmed <i>Gracilaria chilensis</i> by a Pythiosis outbreak
2:45pm – 3:00pm	FRANÇOIS THOMAS - Isotopic tracing reveals single-cell assimilation of macroalgal polysaccharide by marine Flavobacteria and Gammaproteobacteria
Symposium 11	Room "Tali friz"
	Changing distribution patterns and new ecosystems Convenors: Gareth A. Pearson & Anke Kremp
1:30pm – 2:00pm	BENTE EVARDSEN - On the track of causes for fish-killing algal blooms
2:00pm – 2:15pm	*NINA GUÉRIN - In situ and in vitro transcriptomic response of Picophytoplankton <i>Pelagomonas calceolata</i> to low nitrate conditions
2:15pm – 2:30pm	JULIO DE LA ROSA ÁLAMOS - Population dynamics of <i>Ostreopsis</i> cf <i>ovata</i> in Marina del Este, Alboran Sea (Granada, Spain)
2:30pm – 2:45pm	HUGO SCHARFENSTEIN - Comparing the effects of periodic and continuous thermal selection on the fitness of a coral microalgal symbiont
2:45pm – 3:00pm	THIERRY TONON - Pelagic <i>Sargassum</i> events in Jamaica: provenance, morphotype abundance, and biomass composition
Symposium 15 (cont	tinued) Room "Tali melen"
	Algae as producers of valuable compounds Convenors: Abdelfatah Abomohra & Alexei Solovchenko
1:30pm – 2:00pm	INNA KHOZIN-GOLDBERG - Exploring the bioactive potential of microalgae: Sustainable solutions for health and environment
2:00pm – 2:15pm	ALEXEI SOLOVCHENKO - Perspective of microalgae as a bioplatform for the production of inorganic polyphosphates
2:15pm – 2:30pm	HELEN HERBERT - High-value metabolite profiling and bioacitvity potential of four microalgae from distinct lineages

2:30pm – 2:45pm	NICO BETTERLE - Engineering of the recently-discovered <i>Synechococcus</i> PCC 11901, a fast-growing cyanobacteria, for the synthesis of high added-value carotenoids	
2:45pm – 3:00pm	FLORA LAM KIM - Elucidation of phlorotannins with high antioxidant activity in Australian and French fucoids (Phaeophyceae, Fucales)	
3:00pm – 4:25pm	Posters with CoffeeArena Hall(SYM 01, 04, 11, 13, 15)	
4:30pm - 6:00pm	Symposia 01, 04, 11 and 15	
Symposium 01 (contin		
	Taxonomy and Systematics Convenors: Fabio Rindi & Patrick Kociolek	
4:30pm – 4:45pm	LENKA PROCHÁZKOVÁ - Phylogeny and ecophysiology of golden-brown snow algae (Chrysophyceae) from Svalbard, the European Alps and High Tatras	
4:45pm – 5:00pm	CHARLOTTE NIRMA - Chemotaxonomy of holopelagic <i>Sargassum</i> specimens using molecular networking	
5:00pm – 5:15pm	MACKENZIE HEAGY - Mastophoraceae diversity within the Mariana Islands	
5:15pm – 5:30pm	PILAR DÍAZ-TAPIA - Phylogenomic analyses reveal a new introduced species in Europe: <i>Lophurella stichidiosa</i> comb. nov. (Rhodomelaceae, Rhodophyta)	
5:30pm – 5:45pm	CHRISTINE MAGGS - NGS reveals life history of the red alga Nemastoma dichotomum	
5:45pm – 6:00pm	LINE LE GALL - Toward an inventory of marine subantarctic algae: investigations in Kerguelen, Crozet, Saint Paul & Amsterdam	
Symposium 04 (continued) Room "Tali moan"		
	Algae and their intimate partners Convenors: Johan Decelle & Aschwin H. Engelen	
4:30pm – 4:45pm	CAROLINE JUERY - <i>Phaeocystis/Acantherea</i> symbiosis: genomic and transcriptomic insight of core transporter genes	
4:45pm – 5:00pm	KATHERINE HELLIWELL - Characterising the bacterial pathobiome of diatoms inhabiting a productive temperate coastal ecosystem	
5:00pm – 5:15pm	MAËLLE ZONNEQUIN - Metabolic co-evolution: the case of the oxylipin pathway in a host-endophyte interaction between brown macroalgae	
5:15pm-5:30pm	SYLVAIN FAUGERON - Deciphering the domestication of a seaweed holobiont	
5:30pm - 5:45pm	FLORIAN WEINBERGER - A Jekyll-and-Hyde seaweed pathogen	
5:45pm – 6:00pm	ASCHWIN ENGELEN - Kelp microbiomes benefits to the test: bacteria isolation, characterization, and tested for influence on kelp resilience to global change stress conditions	

Symposium 11 (cont	tinued) Room Changing distribution patterns and new ecosystems Convenors: Gareth A. Pearson & Anke Kremp	"Tali friz"
4:30pm – 5:00pm	ESTER SERRÃO - Climate shaping genetic biodiversity of polar ma	rine forests
5:00pm – 5:15pm	JEAN-CHARLES LECLERC - Multi-scale patterns in the structure communities associated with seaweeds in marinas	of fouling
5:15pm – 5:30pm	RAÜL TRIAY-PORTELLA - Niche divergence and species distributities to evaluate the spread of <i>Rugulopteryx okamurae</i> in Europe	ion models
5:30pm – 5:45pm	LJILJANA IVEŠA - The last remaining populations of <i>Gongolar</i> along the Istrian Coast (northern Adriatic)	ia barbata
5:45pm – 6:00pm	ANNALISA FALACE - Species loss and long-term decline in diversity of macroalgae in the northern Adriatic Sea over the last s	
Symposium 15 (continued)       Room "Tali melen"         Algae as producers of valuable compounds       Convenors: Abdelfatah Abomohra & Alexei Solovchenko		
4:30pm – 4:45pm	*FEDERICA DI COSTANZO - Omic approaches for the identit biosynthetic pathways for bioactive compounds in th <i>Thalassiosira rotula</i>	
4:45pm – 5:00pm	SAVERIO SAVIO - A biorefinery approach to extract multiple compounds from the diatom <i>Staurosirella pinnata</i> for drug discov	
5:00pm – 5:15pm	FRANZ GOECKE - A tri-continental survey on the inclusion of mac the diet	croalgae in
5:15pm – 5:30pm	MICHAEL ROSS - Mining <i>Porphyridium cruentum</i> biodiversity for products	high value
5:30pm – 5:45pm	DVIR IRIT - Characterization of emulsions stabilized by polysacch the red microalga <i>Porphyridium</i> sp.	aride from
5:45pm – 6:00pm	VALÉRIE STIGER-POUVREAU - Evolution of thalli and polysacchar of holopelagic <i>Sargassum</i> species during their drift until their stran	- ·
7:00pm – 10:00pm	Public lecture (in French)Room "	Tali ebrel"

# Tuesday 22<sup>nd</sup> August

0.00 6.00	
8:00am – 6:00pm	Registration     Arena Hall
9:00am - 10:00am	Plenary Lecture Room "Tali ebrel" KARIN RENGEFORS
From single cells to	populations – towards understanding phytoplankton diversity and adaptation
10:00am – 11:00am	
10:00am – 11:00am	Symposia 02, 06, 10 and 08
Symposium 02	Room "Tali ebrel"
	iversity assessment and algal distribution in space and time
Convenors: Sophie	Steinhagen & Uwe John co-organised the symposium but cannot attend. The
keynote speak	ers (Petra Novak & Klara Wolff) kindly offered to chair the symposium
10:00am – 10:30am	KLARA WOLF - The hidden flows within species: Phytoplankton population
	dynamics in Arctic assemblages
10:30am – 10:45am	*LAURA SCHWEIBOLD - Synoptic observations of small-scale
	phytoplankton variability based on a citizen science program
10:45am – 11:00am	*VICTOR POCHIC - Photoacclimation in the mixotrophic ciliate
	Mesodinium rubrum: phenotypic variability and implications for red tide
	remote sensing
Symposium 06	Room "Tali moan"
	Algal cell biology and ultrastructure
	Convenors: Zoë Popper & Nils Kröger
10:00am - 10:30am	KLAUS HERBURGER - Cell walls and their remodelling in green algae
10:30am – 10:45am	AHLEM BOUGUERBA-COLLIN - Characterisation of alginate lyases from
10:30am – 10:45am	
10:30am – 10:45am 10:45am – 11:00am	AHLEM BOUGUERBA-COLLIN - Characterisation of alginate lyases from
	AHLEM BOUGUERBA-COLLIN - Characterisation of alginate lyases from brown algae
10:45am – 11:00am	AHLEM BOUGUERBA-COLLIN - Characterisation of alginate lyases from brown algae FRITHJOF KÜPPER - Synchrotron X-rays reveal new insight into halogen and trace metal storage in brown algae
	AHLEM BOUGUERBA-COLLIN - Characterisation of alginate lyases from brown algae FRITHJOF KÜPPER - Synchrotron X-rays reveal new insight into halogen and trace metal storage in brown algae Room "Tali friz"
10:45am – 11:00am	AHLEM BOUGUERBA-COLLIN - Characterisation of alginate lyases from brown algae FRITHJOF KÜPPER - Synchrotron X-rays reveal new insight into halogen and trace metal storage in brown algae
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10:45am – 11:00am Symposium 10	AHLEM BOUGUERBA-COLLIN - Characterisation of alginate lyases from brown algae FRITHJOF KÜPPER - Synchrotron X-rays reveal new insight into halogen and trace metal storage in brown algae Room "Tali friz" Ecology of algal systems Convenors: Florian Weingerber & Petra Visser DEDMER VAN DE WAAL - Ecological stoichiometry of phytoplankton disease
10:45am – 11:00am Symposium 10 10:00am – 10:30am	AHLEM BOUGUERBA-COLLIN - Characterisation of alginate lyases from brown algae FRITHJOF KÜPPER - Synchrotron X-rays reveal new insight into halogen and trace metal storage in brown algae Room "Tali friz" Ecology of algal systems Convenors: Florian Weingerber & Petra Visser
10:45am – 11:00am Symposium 10 10:00am – 10:30am	AHLEM BOUGUERBA-COLLIN - Characterisation of alginate lyases from brown algae FRITHJOF KÜPPER - Synchrotron X-rays reveal new insight into halogen and trace metal storage in brown algae Room "Tali friz" Ecology of algal systems Convenors: Florian Weingerber & Petra Visser DEDMER VAN DE WAAL - Ecological stoichiometry of phytoplankton disease CAROLIN PAUL - Interactive effects of warming and zooplankton grazing on
10:45am – 11:00am <b>Symposium 10</b> 10:00am – 10:30am 10:30am – 10:45am	AHLEM BOUGUERBA-COLLIN - Characterisation of alginate lyases from brown algae FRITHJOF KÜPPER - Synchrotron X-rays reveal new insight into halogen and trace metal storage in brown algae <b>Room "Tali friz"</b> <b>Ecology of algal systems</b> Convenors: Florian Weingerber & Petra Visser DEDMER VAN DE WAAL - Ecological stoichiometry of phytoplankton disease CAROLIN PAUL - Interactive effects of warming and zooplankton grazing on a phytoplankton community of the Central Baltic Sea

Symposium 08	Room "Tali melen"	
Convenors: Conce	Algal photosynthesis, carbon fixation and respiration pción Iñiguez Moreno & Giovanni Finazzi co-organised the symposium but note speaker Benjamin Bailleul & Thomas Lacour kindly offered to chair the symposium	
10:00am - 10:30am	YUSUKE MATSUDA - CO <sub>2</sub> -concentrating mechanism in marine diatoms	
10:30am – 10:45am	CHARLOTTE WALKER - Bestrophin-like proteins play a key role in $HCO_3^-$ transport in the <i>Chlamydomonas</i> $CO_2$ concentrating mechanism	
10:45am – 11:00am	MINORI NIGISHI - Bestrophin-knock out gave an impaired CCM phenotype in marine diatom, <i>Phaeodactylum tricornutum</i>	
11:00am - 11:25am	Coffee BreakArena Hall + Room "Pioka"	
11:30am - 12:30am	Symposia 02, 06, 10 and 08	
Symposium 02 (cont		
Convenors: Sophie S	iversity assessment and algal distribution in space and time Steinhagen and Uwe John co-organised the symposium but cannot attend. The ers (Petra Novak & Klara Wolff) kindly offered to chair the symposium	
11:30am – 11:45am	TI FENG SHAN - Genetic diversity and structure of <i>Undaria pinnatifida</i> populations in China and their genetic relationship with those from other countries	
11:45am – 12:00am	RAFAEL PABLO MARTÍN-MARTÍN - Seascape genomics of <i>Saccharina latissima</i> from the St. Lawrence estuary and gulf (Canada)	
12:00am – 12:15am	SARAH BOLIUS - Sediment archives as time-series of millennial phytoplankton adaption	
12:15am – 12:30am	FRANCESCA NERI - Phytoplankton response to marine heatwaves tendencies in the Northern Adriatic Sea	
Symposium 06 (continued) Room "Tali moan"		
	Algal cell biology and ultrastructure Convenors: Zoë Popper & Nils Kröger	
11:30am – 11:45am	GWANG HOON KIM - ROS signaling in red algae	
11:45am – 12:00am	GINGA SHIMAKAWA - Mapping of subcellular local pHs in marine diatoms	
12:00am – 12:15am	ANDREAS HOLZINGER - 3D reconstruction of <i>Spirogyra</i> and <i>Zygnema</i> (Charophyta) zygospores reveals new details on maturation and cell wall formation	
12:15am – 12:30am	ATSUKO TANAKA - Detailed observation of secondary chloroplast division in <i>Phaeodactylum tricornutum</i>	

Symposium 10 (cont	inued) Room "Tali friz"
	<b>Ecology of algal systems</b> Convenors: Florian Weingerber & Petra Visser
11:30am – 11:45am	LUISA MANGIALAJO - <i>Ostreopsis</i> spp. blooms in relation to macroalgal communities in the framework of regime shifts
11:45am – 12:00am	*ANDREA CASTALDI - Investigating the molecular mechanism of diatom's adhesion using metabolomics to innovate antifouling materials
12:00am – 12:15am	*IRENE ROMERO RODRIGUEZ - Effects of nutrient limitation on survival, infectivity, metabolism and swimming behaviour of <i>Amoebophrya ceratii</i> dinospores
12:15am – 12:30am	*RONNY STEINBERG - Adaption of a non-native <i>Vaucheria</i> species to different light conditions in the Wadden Sea (north-east Atlantic)
Symposium 08 (cont	inued) Room "Tali melen"
	Algal photosynthesis, carbon fixation and respiration pción Iñiguez Moreno & Giovanni Finazzi co-organised the symposium but note speaker Benjamin Bailleul & Thomas Lacour kindly offered to chair the symposium
11:30am – 11:45am	CLARA SIMON - Growth patterns and carbon fluxes: investigation of <i>Ulva</i> spp. primary metabolism using <sup>13</sup> CO <sub>2</sub> -labelling
11:45am – 12:00am	PAMELA FERNÁNDEZ - Local adaptation across multiple life stages and populations: implications for kelps resilience to ongoing climate change
12:00am – 12:15am	RAQUEL SANCHEZ DE PEDRO - Resilience of a warm-temperate fucoid to a simulated marine heatwave: Exploring the interplay between life stages and tissues in relation to emersion
12:15am – 12:30am	KIARA FRANKE - Variable seasonal photosynthetic quotients of a kelp species - A mirror of the complex relationship between oxygen and carbon
12:30am – 1:25pm	Lunch Room "Pioka"
1:30pm – 3:00pm	Symposia 02, 06, 10 and 08
Symposium 02 (continued)Room "Tali ebrel"Biodiversity assessment and algal distribution in space and timeConvenors: Sophie Steinhagen & Uwe John co-organised the symposium but cannot attend. The keynote speakers (Petra Novak & Klara Wolff) kindly offered to chair the symposium	
1:30pm – 1:45pm	JANINA BRAKEL - Exploring patterns of species diversity and species geographic distribution of epiphytic filamentous red algae (EFAs) affecting <i>Kappaphycus</i> and <i>Eucheuma</i> macroalgae
1:45pm – 2:00pm	ANAËLLE BIZIEN - Long-term monitoring of intertidal macroalgal communities: ecological quality assessment and trajectory analysis
2:00pm – 2:15pm	CATIA MONTEIRO - Recent changes in rocky shore biodiversity across the NE Atlantic coast

2:15pm – 2:30pm	MICKAEL LE GAC - Inferring population genetic structure from meta- transcriptomic samples
2:30pm – 2:45pm	JOANA PEREIRA - Major contraction at warmer edges of the distribution of the cold-water algae <i>Himanthalia elongata</i> (Linnaeus) S.F. Gray 1821
2:45pm – 3:00pm	GABRIELA BORER - What is in the turf? A multi-approach biodiversity assessment of turf-forming algae on rocky shores
Symposium 06 (con	tinued) Room "Tali moan"
Symposium oo (con	Algal cell biology and ultrastructure Convenors: Zoë Popper & Nils Kröger
1:30pm – 2:00pm	ASSAF GAL - Using cryo electron microscopy to study diatom silicification
2:00pm – 2:15pm	*ALESSANDRA PETRUCCIANI - Cell wall evolution in diatoms: how are shape, size and silicification involved in frustule adaptive functions?
2:15pm – 2:30pm	*MARTIM CARDADOR - Morphological and physiological remodelling of <i>Chlamydomonas reinhardtii</i> zygotes during zygospore formation
2:30pm – 2:45pm	MOHAMED GHOBARA - Light focusing in pennate Diatoms: Can living diatoms see?
2:45pm – 3:00pm	GLEN WHEELER - Understanding calcium signalling pathways in diatom chloroplasts in response to high light and oxidative stress
Symposium 10 (con	tinued) Room "Tali friz"
	<b>Ecology of algal systems</b> Convenors: Florian Weingerber & Petra Visser
1:30pm – 2:00pm	MARINE VALLET - Chemical signaling in the algal holobiont
2:00pm – 2:15pm	LUIS FERREIRA PEREIRA - Geographical variability of physiological responses of <i>Ascophyllum nodosum</i> to realistic simulations of intertidal conditions in a self-contained autonomous system
2:15pm – 2:30pm	MARK JOHNSON - Peak fucoid canopy photosynthesis in space and time
2:30pm – 2:45pm	CLÉMENCE BOUCHER - <i>Prymnesium parvum</i> , a bloom-forming haptophyte that use toxins to mediate food uptake, especially under P-limitation
2:45pm – 3:00pm	CLÉMENCE BLAIS - Selective feeding of constitutive mixotrophic dinoflagellates in response on different quality prey
Symposium 08 (con	tinued) Room "Tali melen"
	Algal photosynthesis, carbon fixation and respiration
Convenors: Concepción Iñiguez Moreno & Giovanni Finazzi co-organised the symposium but cannot attend. keynote speaker Benjamin Bailleul & Thomas Lacour kindly offered to chair the symposium	
1:30pm – 1:45pm	MANA NAKAYASU - The role of Lhcx isoforms in photoprotection mechanism in the diatom, <i>Thalassiosira pseudonana</i>
1:45pm – 2:00pm	CHIARA GIOSSI - The violaxanthin cycle can provide non-photochemical quenching in <i>Phaeodactylum tricornutum</i>

2:00pm – 2:15pm	MARTIN LAVIALE - Effect of heat stress on phototrophic biofilm photosynthesis in the context of climate change: a thermal dose approach	
2:15pm – 2:30pm	SILJA FRANKENBACH - Photoinactivation vs repair of photosystem II under thermal stress in epipelic and epipsammic microphytobenthos communities	
2:30pm – 2:45pm	JEROME MORELLE - Motility of epipelic diatoms: inter- and intra-specific variability of its photoprotective role under a realistic light microgradient	
2:45pm – 3:00pm	ERIK JENSEN - Using <i>Cyclotella cryptica</i> as a new model to understand the physiological, molecular and genetic regulation of trophic mode switching in facultative heterotrophic diatoms	
3:00pm – 4:25pm	Posters with CoffeeArena Hall(SYM 02, 06, 08, 10)	
4:30pm - 6:00pm	Symposia 02, 06, 10 and 08	
Symposium 02 (continued)Room "Tali ebrel"Biodiversity assessment and algal distribution in space and timeConvenors: Sophie Steinhagen & Uwe John co-organised the symposium but cannot attend. The keynote speakers (Petra Novak & Klara Wolff) kindly offered to chair the symposium		
4:30pm – 4:45pm	YOAV AVRAHAMI - In-depth characterization of diatom community succession over marked seasonal transitions in the subtropical Gulf of Aqaba- Eilat	
4:45pm – 5:00pm	FIDDY SEMBA PRASETIYA - Unveiling the hidden diversity of epiphytic diatoms associated with macroalgae <i>Padina</i> in the Indonesian Waters	
5:00pm – 5:15pm	LUIS BARRETO - Marine heatwave susceptibility at the trailing edge of the foundational kelp, <i>Laminaria hyperborea</i>	
5:15pm – 5:30pm	MAGGIE REDDY - Integrating metabolomics and DNA to uncover patterns of diversity in two sister species of kelp	
5:30pm – 5:45pm	MARINA MONTRESOR - Interannual variability of genetic and demographic structure in blooms of the marine diatom <i>Pseudo-nitzschia multistriata</i>	
5:45pm – 6:00pm	LINDA MEDLIN - Automated environmental sampler and laboratory-on-a-chip to enhance monitoring for toxic algae	
Symposium 06 (contin	nued) Room "Tali moan"	
	Algal cell biology and ultrastructure Convenors: Zoë Popper & Nils Kröger	
4:30pm – 4:45pm	ELLEN HARRISON - Mixed messages: The influence of nitrate and phosphate limitation on nutrient sensing in model marine diatoms	
4:45pm – 5:00pm	MAXIM BOKOV - Nitrogen fixation in the form of guanine crystals and rearrangements of crystals as an adaptation for various illumination levels of <i>Amphidinium carterae</i>	
5:00pm – 5:15pm	ALESSANDRA ROGATO - Diatoms nitrate transporters as regulators of intracellular nitrogen dynamics in a changing environment	

5:15pm – 5:30pm	JUSTIN LAU - The phase separated CO <sub>2</sub> -fixing <i>Chlamydomonas</i> pyrenoid proteome determined by TurboID	
5:30pm – 5:45pm	NILS KRÖGER - An intimate view into the silica deposition vesicle of the model diatom <i>Thalassiosira pseudonana</i>	
5:45pm – 6:00pm	PETER KROTH - Aureochromes - Unique photoreceptors in diatoms	
Symposium 10 (cont	tinued) Room "Tali friz"	
	<b>Ecology of algal systems</b> Convenors: Florian Weingerber & Petra Visser	
4:30pm – 4:45pm	OLATZ ARRIAGA - Cornered by turfs: The alarming decline of forest-like macroalgal communities in the south-eastern Bay of Biscay	
4:45pm – 5:00pm	GUILLAUME MANAT - What are you doing here? A surprising beta- porphyranase activity in a carrageenophyte red alga	
5:00pm – 5:15pm	CHARLOTTE NEF - Global survey of vitamin B12-related genes in phytoplankton reveals contrasting metabolic abilities between communities	
5:15pm – 5:30pm	LARA PRELLE - Coastal peatland water stimulates growth of Baltic Sea and peatland benthic diatoms	
5:30pm – 5:45pm	INKA BARTSCH - Mediterranean deep water kelp <i>Laminaria rodriguezii</i> is living at optimum temperatures for growth but at the edge of light	
5:45pm – 6:00pm	MYRIAM VALERO - Sexual selection in seaweed ? The case of the red alga <i>Gracilaria gracilis</i>	
Symposium 08 (continued)       Room "Tali melen"         Algal photosynthesis, carbon fixation and respiration         Convenors: Concepción Iñiguez Moreno & Giovanni Finazzi co-organised the symposium but cannot attend. keynote speaker Benjamin Bailleul & Thomas Lacour kindly offered to chair the symposium		
4:30pm – 4:45pm	*LINDA REHDER - Abrupt and acclimation responses to changing temperature elicit divergent physiological effects in the diatom <i>Phaeodactylum tricornutum</i>	
4:45pm – 5:00pm	*ANXHELA HANIA - A multi-level approach to investigate the dynamics and spatial heterogeneity of N <sub>2</sub> fixation and photosynthesis in <i>Trichodesmium</i>	
5:00pm – 5:15pm	*DANY CROTEAU - Titrating nonphotochemical quenching to develop a comprehensive model of photosynthesis regulation in the diatom <i>Phaeodactylum tricornutum</i>	
5:15pm - 5:30pm	ONYOU NAM - Identifying the proteins underpinning the diatom pyrenoid	
5:30pm – 5:45pm	GRAHAM UNDERWOOD - The influence of light spectra, intensity and photosynthesis on the motility behaviour and photoacclimation of two species of benthic diatoms	

5:45pm – 6:00pm	THOMAS LACOUR - Dynamic and sustained non-photoche under light/dark illumination and nutrient starvation in p culture and in natural communities	1 0
6:00pm – 7:30pm	Silent Auctions organized by the French Phycological Society (SPF) with Food and Drink	Room "Pioka"

# Wednesday 23<sup>rd</sup> August

8:00am - 5:00pm	Excursions	
9:00am – 1:00pm	Workshops	Faculty of Letters - UBO
	<b>.</b> .	Room A219 <b>: present advances and future challenges</b> r, Joao Serodio, Koen Sabbe & Graham Underwood
GRAHAM UNDERWOO microphytobenthic eco		the bottom up, how species attributes influence
DAVID PATERSON - M	icrophytobenthos respon	nse to multiple stressors
CAROLINE DOOSE - O	xylipines synthesis in m	icrophytobenthos
ANNA ISAIA - Photoph	ysiology of a subtidal e	pipelic diatom in the Bay of Brest
DORIAN BAS - Multi- euglenophyte dominat	*	ity in MPB biomass and production in diatom- and
JÉRÔME MORELLE - microgradients	Motility and photosy	nthetic performance of epipelic diatoms in light
MARTIN LAVIALE - E climate change: a ther		phototrophic biofilm photosynthesis in the context of
	ANG - Mapping intertida D2 fluxes: from meso- to	l mudflat primary production by coupling hyperspectral macroscale
Blitz Talk - LOURENG based biotic index in t	•	he analysis as a steppingstone towards a new diatom-
1h Discussion		
9:00am – 1:00pm Workshop 2 Room A214 Getting together to fight the new invasive alien seaweed: <i>Rugulopteryx okamurae</i> (Dictyotales, Ochrophyta) Organiser: María Altamirano		
	- Rugulopteryx okamun cern and what we know	<i>tae</i> (Dictyotales, Ochrophyta): the first invasive alien about it in Spain
FATIMA EL AAMRI - Moroccan coast	Current status of the	invasive brown alga Rugulopteryx okamurae on the

JOÃO FARIA - Following the invader *Rugulopteryx okamurae* in Azores (NE Atlantic): challenges and perspectives

MARIE BORRIGLIONE - Dynamic and impact of *Rugulopetryx okamurae* along the Mediterranean French coast

SABIN LIULEA - Spread of the invasive brown alga Rugulopteryx okamurae in Portugal (NE Atlantic)

ALEJANDRO BERNAL-IBÁÑEZ - Integrative approach assessing *Rugulopteryx okamurae* invasion at Madeira

RICARDO HAROUN - Arrival and early spread of Rugulopteryx okamurae in the Canary Islands

#### 2h Round Table

9:00am – 1:00pm	Workshop 3	Room A221
Add	ressing fundamental gaps in seaweed taxonomic knowledge	
	Global Seaweed Biodiversity Group	
	Organisers: Juliet Brodie & Rob Mrowicki	
9:00am – 1:00pm	Workshop 4	Room A220
_	The 100 Diatom Genomes project	
	Organiser: Thomas Mock	
9:00am – 1:00pm	Workshop 5	Room A223
Exchange around drifting Sargassum species: fundamental and applied research		
	Organiser: Valérie Stiger-Pouvreau	

### Thursday 24<sup>th</sup> August

8:00am - 10:00am	Registration   Arena Hall
9:00am - 10:00am	Plenary Lecture Room "Tali ebrel"
	ANGELA FALCIATORE
	Light in the life of marine diatoms
10:00am - 11:00am	Symposia 03, 04, 09 and 16
Symposium 03	Room "Tali ebrel"
	<b>Phylogenetics, Phylogenomics and Evolution</b> Convenors: Yvonne Němcová & Olivier de Cleck
10:00am – 10:30am	BOJIAN ZHONG - Phylotranscriptomics unveil a Paleoproterozoic- Mesoproterozoic origin and deep relationships of the Viridiplantae
10:30am – 10:45am	*CAMILLE PUGINIER - Phylogenomics reveals the evolutionary origin of lichenization in chlorophyte algae
10:45am – 11:00am	ROSA CELIA POQUITA-DU - Genome-wide comparisons reveal extensive divergence within the common lichen photobiont genus, <i>Trebouxia</i>

Symposium 04	Room "Tali moan" Algae and their intimate partners
	Convenors: Johan Decelle & Aschwin H. Engelen
10:00am – 10:30am	SHADY AMIN - Vitamin B6 derivatives mediate symbiotic exchanges between microalgae and bacteria
10:30am – 10:45am	MATTHIEU GARNIER - To what extent and how does the phytoplankton choose their bacterial partners. A reductionist approach used in toxic microalgae
10:45am – 11:00am	FLORA VINCENT - Viral impacts across biological scales: from single-cells to planktonic ecosystems
Symposium 09	Room "Tali friz" <b>The global carbon-cycle</b> Convenors: Karen Filbee-Dexter & Sebastian Rokitta
10:00am – 10:30am	KAREN FILBEE-DEXTER - Carbon export from seaweed forests to deep ocean sinks
10:30am – 10:45am	MARC LONG - Investigating the effects of allelopathy on organic matter sink during microalgal blooms
10:45am – 11:00am	ANTONIJA MATEK - Modelling primary production at Lastovo Island (South Adriatic Sea)
Symposium 16	Room "Tali melen"
C	Advances in algal production processes Convenors: Maria do Rosario Domingues & Joanna Kargul
10:00am – 10:30am	HUGO PEREIRA - Improving the sustainability of algae production in commercial-scale facilities
10:30am – 10:45am	ZOFIA NEHR - Strain selection in Ulva spp. for land-based aquaculture
10:45am – 11:00am	CLEMENT GAUCI - Priming as a kelp crop-enhancement strategy for seaweed cultivation under environmental challenges
11:00am – 11:25am	Coffee Break Arena Hall + Room "Pioka"
11:30am – 12:30am	Symposia 03, 05, 09 and 16
Symposium 03 (conti	inued) Room "Tali ebrel" Phylogenetics, Phylogenomics and Evolution Convenors: Yvonne Němcová & Olivier de Cleck
11:30am – 11:45am	HEROEN VERBRUGGEN - The chloroplast genome of early diverging Cladophorales represents the missing link to hairpin chromosomes
11:45am – 12:00am	ALEXANDER BOWLES - Algal metagenomics provides insights into land plant terrestrialisation
12:00am – 12:15am	EL MAHDI BENDIF - Cryptic diversification in a widespread phytoplankton at low latitudes

12:15am – 12:30am	HELENA CRUZ DE CARVALHO - Shedding light on the noncoding transcriptome of the ocean plankton	
Symposium 05       Room "Tali moan"         New insights into the mechanisms and regulation of life cycles in algae       Convenors: Marielle Ferrante & Agnieszka Lipinska		
11:30am – 12:00am	GUST BILCKE - Multi-species comparative -omics reveals marker genes for the diatom life cycle	
12:00am – 12:15am	FRANCISCO J. ROMERO-CAMPERO - Multiomics responses to seasonal variations in diurnal cycles in the marine picoeukaryote Ostreococcus tauri	
12:15am – 12:30am	SAMUEL BOSCQ - Role of the maternal tissue in the establishment of polarity axes in the early embryo of the brown alga <i>Saccharina latissima</i>	
Symposium 09 (cont	inued) Room "Tali friz"	
v t x	The global carbon-cycle Convenors: Karen Filbee-Dexter & Sebastian Rokitta	
11:30am – 12:00am	MAR FERNANDEZ MENDEZ - The role of holopelagic <i>Sargassum</i> in the marine carbon cycle and its potential for carbon dioxide removal	
12:00am – 12:15am	*INGA HELLIGE - Brown algal fucoidan secretion and stability around the globe	
12:15am – 12:30am	MENG ZHANG - Mapping intertidal mudflat primary production by coupling hyperspectral remote sensing and CO <sub>2</sub> fluxes: from meso- to macroscale	
Symposium 16 (continued) Room "Tali melen"		
Advances in algal production processes Convenors: Maria do Rosario Domingues & Joanna Kargul		
11:30am – 12:00am	YAGUT ALLAHVERDIYEVA-RINNE - Green chemistry through photosynthetic microbial biocatalysts	
12:00am – 12:15am	SVETLANA VASILIEVA - Bio-inspired materials for increasing the polyunsaturated fatty acids productivity of microalgae: <i>Lobosphaera</i> immobilized on polyethylenemine and chitosan-based carriers	
12:00am – 12:15am 12:15am – 12:30am	polyunsaturated fatty acids productivity of microalgae: Lobosphaera	
	<ul><li>polyunsaturated fatty acids productivity of microalgae: Lobosphaera immobilized on polyethylenemine and chitosan-based carriers</li><li>ASWIN SASI - Effect of an optical lightguide on growth of Arthrospira</li></ul>	
12:15am – 12:30am	polyunsaturated fatty acids productivity of microalgae: <i>Lobosphaera</i> immobilized on polyethylenemine and chitosan-based carriers ASWIN SASI - Effect of an optical lightguide on growth of <i>Arthrospira platensis</i>	
12:15am – 12:30am 12:30am – 1:25pm	polyunsaturated fatty acids productivity of microalgae: Lobosphaeraimmobilized on polyethylenemine and chitosan-based carriersASWIN SASI - Effect of an optical lightguide on growth of ArthrospiraplatensisLunchRoom "Pioka"Symposia 03, 05, 20 and 14	

<ul> <li>2:00pm – 2:15pm</li> <li>OZAN CIFTCI - Phylotranscriptomics reveals the reticulate evolut history of a widespread diatom species complex</li> <li>2:15pm – 2:30pm</li> <li>ANANYA KHATEI - Presence and evolution of DNA methyltransferas associated domains in brown algal genomes</li> <li>2:30pm – 2:45pm</li> <li>RICHARD DORRELL - Fucoxanthin-containing dinoflagellates: An experiment in plastid proteome evolution</li> <li>2:45pm – 3:00pm</li> <li>MARIE-LAURE GUILLEMIN - Sequencing of seaweeds ancient DNA</li> </ul>	open	
associated domains in brown algal genomes 2:30pm – 2:45pm RICHARD DORRELL - Fucoxanthin-containing dinoflagellates: An experiment in plastid proteome evolution	open from	
experiment in plastid proteome evolution	A from	
2:45pm – 3:00pm MARIE-LAURE GUILLEMIN - Sequencing of seaweeds ancient DNA		
Monte Verde, one of the oldest archeological sites from South Americ		
Symposium 05 (continued) Room "Tali	moan"	
New insights into the mechanisms and regulation of life cycles in algae Convenors: Marielle Ferrante & Agnieszka Lipinska		
1:30pm – 2:00pm KENNY BOGAERT - Zygote (a)symmetric cell division in <i>Dicty</i> developmental hinge point	<i>ota</i> : a	
2:00pm – 2:15pm *LAURIE BOUSQUET - Transcriptome differentiation between life of the bloom forming marine phytoplankton specie <i>Emiliania huxi</i>	-	
2:15pm – 2:30pm *PÉLAGIE RATCHINSKI - Developmental and evolutionary surv gene expression during brown algal life cycles	eys of	
2:30pm – 2:45pm DANIEL LIESNER - Unveiling mechanisms of sexual differentiation feminized males of the giant kelp <i>Macrocystis pyrifera</i>	using	
2:45pm – 3:00pm LÉA PRIGENT - Sexual reproduction, a major event determining the dyn of gene expression during a <i>P. australis</i> bloom?	namics	
Symposium 20 Room "Ta	li friz"	
Coastal and freshwater systems under human pressure: status assessment, managemen	t and	
conservation		
Convenors: Due to the passing away of our colleague Erwan Ar Gall, keynote speaker Elvira Ramos kindly offered to prepare and chair the symposium with Sotiris Orfanidis		
1:30pm – 2:00pm       MATINA KATSIAPI - Assessing ecological water quality of Mediter lakes/reservoirs using phytoplankton indices	ranean	
2:00pm – 2:15pm PATRICIJA MOZETIČ - How the ongoing oligotrophication of the ne Adriatic affects phytoplankton community structure and functioning	orthern	
2:15pm – 2:30pm GIULIA CHELONI - Organic contaminants fate in aquatic ecosystems, role for microalgae?	which	
2:30pm – 2:45pm LOURENÇO RIBEIRO - Using niche analysis as a steppingstone towards diatom-based biotic index in transitional waters	a new	
2:45pm – 3:00pm MARTIN PLUS - Biogeochemical modelling of eutrophication in waters. An ensemble model approach for the calculation of threshold l		

Symposium 14 Algal culture coll	Room "Tali melen" ections: their increasing societal relevance and role as gene repositories Convenors: Filip Pniewski & Peter Chaerle
1:30pm – 2:00pm	ANNICK WILMOTTE - Microalgal culture collections : their roles and challenges in the $21^{th}$ century
2:00pm – 2:15pm	JOSEPH KIHIKA - Cryopreservation of diverse Symbiodiniaceae species: fatty acid profiles, growth rates and photosynthetic performance
2:15pm – 2:30pm	MAIKE LORENZ - Algal culture collections in a changing world
2:30pm - 3:00pm	DANIEL VAULOT -
3:00pm – 4:25pm	Posters with Coffee         Arena Hall           (SYM 03, 07, 09, 12, 14, 16, 17, 18+19, 20)         Arena Hall
4:30pm - 5:30pm	Symposia 03, 05, 20 and 08
Symposium 03 (cont	inued) Room "Tali ebrel" Phylogenetics, Phylogenomics and Evolution Convenors: Yvonne Němcová & Olivier de Cleck
4:30pm – 4:45pm	AURÉLIEN BAUD - Uncovering local adaptation to temperature process in the red seaweed <i>Palmaria palmata</i> intertidal populations
4:45pm – 5:00pm	SIGNE BRÅTELUND - Fine-scale genetic structure of sugar kelp populations at short geographical distance
5:00pm – 5:15pm	GARETH PEARSON - Genus-wide transcriptional responses to short-term warming in eastern Atlantic <i>Laminarias</i> (Phaeophyceae, Laminariales): a triumph of phylogeny over phenotype?
5:15pm – 5:30pm	AGNIESZKA LIPINSKA - Scarlet tide - the first report of sex chromosomes in red algae
Symposium 05 (continued)Room "Tali moan"New insights into the mechanisms and regulation of life cycles in algae Convenors: Marielle Ferrante & Agnieszka Lipinska	
4:30pm – 4:45pm	DARJA BELISOVA - Transcriptional regulation of sexual maturation in Cylindrotheca closterium
4:45pm – 5:00pm	EUNYOUNG SHIM - $Ca^{2+}$ signaling during fertilization in the red alga <i>Bostrychia moritziana</i> : the role of calcium-dependent kinases in ROS signaling
5:00pm – 5:15pm	MARIELLA FERRANTE - Genetic and epigenetic controls governing life cycle transitions in the planktonic diatom <i>Pseudo-nitzschia multistriata</i>
5:15pm – 5:30pm	STACY KRUEGER-HADFIELD - Isomorphic may not be so isomorphic after all

Symposium 20 (continued)Room "Tali friz"Coastal and freshwater systems under human pressure: status assessment, management and conservationConvenors: Due to the passing away of our colleague Erwan Ar Gall, keynote speaker Elvira Ramos kindly offered to prepare and chair the symposium with Sotiris Orfanidis		
4:30pm – 5:00pm	ELVIRA RAMOS - Macroalgal monitoring challenges under t community changes	he climate-driven
5:00pm – 5:15pm	ANNETTE BRUHN - Cultivating sugar kelp as an instrume eutrophication	ent for mitigating
5:15pm – 5:30pm	SOTIRIS ORFANIDIS - Photosynthetic and growth responses of to survive the fluctuating conditions in eutrophic Thessalon	
Symposium 08 (continued)         Room "Tali melen"           Algal photosynthesis, carbon fixation and respiration           Convenors: Concepción Iñiguez Moreno & Giovanni Finazzi co-organised the symposium but cannot attend. keynote speaker Benjamin Bailleul & Thomas Lacour kindly offered to chair the symposium		
4:30pm – 5:00pm	BENJAMIN BAILLEUL - Measurements of photosynthetic mixtures reveal biotic interactions between diatoms and dim	
5:00pm – 5:15pm	JHOANELL ANGULO - Comparative analysis of photosystrategies to Fe limitation in the marine diatoms <i>Thalassia</i> and <i>Thalassiosira oceanica</i>	-
5:15pm – 5:30pm	PASCAL CLAQUIN - Five years of high frequency FRRF me the SMILE buoy - Estimation of primary production from h	
7:00pm – 11:00pm	Congress Diner	Les Capucins

# Friday 25<sup>th</sup> August

9:00am - 10:00am	Plenary Lecture       Room "Tali ebrel"         THOMAS WERNBERG       Kelp forests in the Anthropocene
10:00am - 11:00am	Symposia 17, 07, 12 and 18+19
Symposium 17	Room "Tali ebrel" <b>Public-Private partnerships in algal research</b> Convenors: Stefan Kraan & Annette Bruhn
10:00am – 10:30am	ESBEN CHRISTIANSEN - 3 phases towards an industrial land-based seaweed cultivation
10:30am – 10:45am	ALEXANDER JUETERBOCK - Roadmap towards sustainable growth of the European seaweed industry

10:45am – 11:00am	ROY DURLAVE - Studies on the use of locally available (Coxs Bazar and Saint Martin) alternative renewable seaweed wastes as compost organic fertilizer resources
Symposium 07	Room "Tali moan" <b>Genomics technologies in algae</b> Convenors: Maria Mittag & Claire Gachon
10:00am – 10:30am	THOMAS MOCK - Genomics and reverse genetics in diatoms
10:30am – 10:45am	SERENA ROSIGNOLI - The genome of <i>Palmaria palmata</i> : a tool to advance seaweed strain selection and unveil intraspecific variability across Northern Europe
10:45am – 11:00am	ANNE M. L. NILSEN - Chloroplast DNA methylation in the kelp <i>Saccharina latissima</i> is determined by origin and influenced by cultivation
Symposium 12	Room "Tali friz"
	<b>Polar algae in a changing environment</b> Convenors: Linda Nedbalová & Johann Lavaud
10:00am – 10:30am	CHRIS WILLIAMSON - Biosphere - Cryosphere interactions during periods of rapid environmental change
10:30am – 10:45am	*CLARENCE WEI HUNG SIM - Under-ice bloom: temporal dynamics and biogeography of Arctic sympagic algae and phytoplankton
10:45am – 11:00am	*HELEN FEORD - Single-cell elemental profiling of polar snow algae to investigate cellular responses to low nutrient availability in the cryosphere
Symposium 18 + 19	Room "Tali melen"
	Algae and the general public Convenors: Nils Ekelund & Johanna Weggelar
C	Algae, education and career pathways onvenors: Cecilia Maria Totti & Conxi Rodríguez-Prieto
10:00am – 10:30am	MORGAN VIS - Mentoring undergraduate and graduate students for careers inside and outside academia
10:30am – 10:45am	VICKY DOMINGUEZ ALMELA - Developing citizen science monitoring capacity for adaptation to brown seaweed influxes
10:45am – 11:00am	JOSÉ LUCAS PÉREZ-LLORÉNS - Phycophagy and the rise of the phycogastronomy in Europe
11:00am – 11:25am	Coffee Break Arena Hall + Room "Pioka"
11:30am – 12:30am	Symposia 17, 07, 12 and 18+19

Symposium 17 (continued)Room "Tali ebrel"Public-Private partnerships in algal research Convenors: Stefan Kraan & Annette BruhnRoom "Tali ebrel"		
11:30am – 12:00am	MONIQUE MULDER - Discovery of the potential of brown seaweed lipid extracts against Alzheimer's disease in a public-private partnership	
12:00am – 12:15am	AMANDYNE LINARES-MAURIZI - Valorization of marine oxylipins; a story of private-public partnership	
12:15am – 12:30am	ANNETTE BRUHN - Algaecenter Denmark – from research to industry	
Symposium 07 (continued)       Room "Tali moan"         Genomics technologies in algae       Convenors: Maria Mittag & Claire Gachon		
11:30am – 12:00am	SIGRID NEUHAUSER - Phytomyxea use conserved host physiological properties to induce a sink and to avoid host defence	
12:00am – 12:15am	RISHIRAM RAMANAN - Carbon-neutral, eco-friendly polymer from microalgae and cyanobacteria	
12:15am – 12:30am	CAROLE DUCHÊNE - Diatom phytochromes: solving the riddle of a red-light sensor in the red-poor marine environment	
Symposium 12 (continued)       Room "Tali friz"         Polar algae in a changing environment       Convenors: Linda Nedbalová & Johann Lavaud		
11:30am – 11:45am	*NORA DIEHL - Heading northwards? <i>Laminaria hyperborea</i> in the Arctic - past, present and future	
11:45am – 12:00am	RAQUEL CARMONA - Seasonal changes in photosynthesis and biochemical composition in Arctic macroalgae undergoing a climatic transition	
12:00am – 12:15am	PAMELA FERNÁNDEZ - Inorganic carbon uptake strategies in Antarctic seaweeds: implications for seaweed resilience to ocean acidification	
12:15am – 12:30am	LUISA DÜSEDAU - Influence of recent warming on kelp forest structure in Kongsfjorden (western Svalbard) over time	
Symposium 18 + 19 (continued) Room "Tali melen"		
Algae and the general public Convenors: Nils Ekelund & Johanna Weggelar		
Algae, education and career pathways Convenors: Cecilia Maria Totti & Conxi Rodríguez-Prieto		
11:30am – 12:00am	ALIX LEVAIN - When Algae go public: revenge of the margins?	
12:00am – 12:30am	FREDRIK GRÖNDAHL - The importance with outreach in order to promote interest and use of seaweeds in a Swedish and European context	
12:30am – 1:25pm	Lunch Room "Pioka"	
1:30pm – 2:30pm	Symposia 02, 12, 18+19 and a Special Session	

Symposium 02 (continued)Room "Tali ebrel"Biodiversity assessment and algal distribution in space and timeConvenors: Sophie Steinhagen & Uwe John co-organised the symposium but cannot attend. The keynote speakers (Petra Novak & Klara Wolff) kindly offered to chair the symposium			
1:30pm – 2:00pm	PETRA NOWAK - Environmental DNA-based assessment of Charophyte biodiversity: Progress and remaining challenges		
2:00pm – 2:15pm	FABIO RINDI - Coralline red algae of Antarctica and Subantarctic regions: state of knowledge of a threatened, yet poorly known, group of ecosystem engineers		
2:15pm – 2:30pm	JOANNA WILBRAHAM - A red list for British seaweeds		
1:30pm – 2:30pm       Special Session       Room "Tali moan"         Publishing phycological papers in a rapidly changing environment       Convenors: Juliet Brodie & Christine Maggs			
Symposium 12 (continued)       Room "Tali friz"         Polar algae in a changing environment       Convenors: Linda Nedbalová & Johann Lavaud			
1:30pm – 2:00pm	MARINA CVETKOVSKA - Distant, yet the same: How do Arctic and Antarctic green algae cope with multiple stressors?		
2:00pm – 2:15pm	SEBASTIAN ROKITTA - Arctic <i>Phaeocystis pouchetii</i> benefits stronger from warming than from changes in light intensity or pCO <sub>2</sub>		
2:15pm – 2:30pm	SÉBASTIEN GUÉRIN - Challenged growth and performance across changing Arctic seasonal photoperiods in the polar diatom <i>Fragilariopsis cylindrus</i>		
Symposium 18 + 19 (continued)       Room "Tali melen"         Algae and the general public       Convenors: Nils Ekelund & Johanna Weggelar			
Algae, education and career pathways Convenors: Cecilia Maria Totti & Conxi Rodríguez-Prieto			
1:30pm – 1:45pm	ALESSANDRA NORICI - One-on-one and small	group mentoring in phycology	
1:45pm – 2:00pm	STEFAN KRAAN - Seaweed hype, social media education to sway the opinion of the general public		
2:00pm – 2:30pm	SUZANNE FREDERICQ - What can the Gulf of M education and outreach?	lexico and Panama tell us about	
2:30pm – 2:55pm	Coffee break	Arena Hall + Room "Pioka"	
3:00pm-4:00pm	Feed-back: adressing topics for the future	Room "Tali ebrel"	
4:00 pm - 5:00 pm	<b>Closing Ceremony + Prices</b>	Room "Tali ebrel"	

# **Posters**

All posters will be displayed throughout the Congress in the Hall of Brest Arena. Presenters should be standing beside their posters on the day and time indicated for the Poster session. A code for poster installation is indicated (PO...).

### Monday 21st August – 3:00pm to 4:25pm

SYMPOSIUM 01 "TAXONOMY AND SYSTEMATICS"

**PO1\_A** - ANA BARICEVIC - Brijuni National Park (Adriatic Sea, Croatia) phytoplankton biodiversity assessment

PO2\_A - SÉBASTIEN COLIN - Which microscope to take for field work

**PO3\_A** - JOANNA CZERWIK-MARCINKOWSKA - *Ulva pilifera* (Ulvales, Chlorophyta) from Montenegro – application of X-ray fluorescence techniques in determining the habitat preferences

**PO4\_A** - ANNA FRICKE - A study on the variation of nutritional profiles of seaweeds along the Senegalese coast

**PO5\_A** - BRIGITTE GAVIO - *Dictyota psychedelica* sp. nov., a new prostrate member of the genus in the Caribbean Sea

**PO6\_A** - SVENJA HEESCH - Cryptic diversity in the Prasiolaceae (Prasiolales, Trebouxiophyceae, Chlorophyta)

**PO7\_A** - MATHIEU HÉLIAS - Diversity of the freshwater macroalgae from Guadeloupe, French Lesser Antilles

PO8\_A - JINSOON PARK - A study of marine benthic diatoms from Ulleungdo and Dokdo, Korea

**PO9\_A** - VIVIANA PEÑA - Coralline algae (Rhodophyta, Corallinales) early colonizers of the littoral lava flows of the recent volcanic eruption of La Palma (Canary Islands)

**PO10\_A** - CONXI RODRÍGUEZ-PRIETO - Revisiting the systematics of the *Grateloupia*-complex (Halymeniales, Rhodophyta) based on cystocarp development and molecular phylogeny

**PO11\_A** - FLORENCE ROUSSEAU - Contribution to the taxonomy of *Sargassum* with specimens from French West Indies and south of the Madagascar coasts

**PO12\_A** - SVATOPLUK SKOUPÝ - The application of population genomics in the taxonomy of cyanobacteria

**PO13\_A** - JOANNA WILBRAHAM - A genomic approach towards resolving the taxonomy and phylogenetic relationships of historic stonewort (Charophyta) collections

PO14\_A - JOANNA WILBRAHAM - Diversity and distribution of desmids in Britain and Ireland

**PO15\_A** - GABRIELLE ZAMMIT - DNA barcoding and chemical analysis of algae from Malta in the central Mediterranean

### SYMPOSIUM 04 "ALGAE AND THEIR INTIMATE PARTNERS"

**PO16\_A** - LEONARDO BRUNI - Searching *Phaeobacter* spp. in *Ulva* spp. microbiota: a metadataanalysis

**PO17\_A** - ANNE-SOPHIE BURLOT - Quantitative study of the microbial communities living on the surface of the green seaweed *Ulva sp.* 

**PO18\_A** - GONZALO DEL OLMO - Does light affect the presence of *Phaeobacter* in the *Ulva ohnoi* holobiont by modifying bacterial communities?

**PO19\_A** - GAUTIER DEMOULINGER - Chemical exploration of the *Ascophyllum nodosum* holobiont

**PO20\_A** - ASCHWIN H. ENGELEN - The bacteriome of *Porphyra umbilicalis* in the North-Atlantic across time and space

**PO21\_A** - PAUL ESTOUP - Investigating bacterial diversity of the ulva holobiont in industrial cultures

**PO22\_A** - ELIZABETH FICKO-BLEAN - Marine microbial protein-carbohydrate interactions with red algal molecules

**PO23\_A** - ERIC FOUILLAND - Role of bacteria in the allelopathic ability of an autotrophic biofilm

**PO24\_A** - RKIA LBOUHMADI - Production of fatty acids rich in DHA and EPA from microalgae to enrich infant growth milk

**PO25\_A** - PEDRO MURÚA - Toward an Antarctic phycopathology: description of natural pathosystems associated to the green alga *Urospora penicilliformis* 

**PO26\_A** - LAURA PAGEAULT - Characterization of Cell Cycle Events Affected by Light dose in the microalgae *Tisochrysis lutea* 

**PO27\_A** - HANA PARK - ROS is important in the life cycle and during infection of *Pythium porphyrae*, the causative agent of red rot disease

**PO28\_A** - SAUVANN PAULINO - Interactions between *Ulva* sp. and its cultivable epiphytic bacteria

**PO29\_A** - JOSÉ PINTADO - Shedding light on the *Ulva* holobiont: the role of light in interactions with *Phaeobacter* bacteria

 $PO30\_A$  - FANÇOIS THOMAS - Seasonal dynamics of macroalgae-associated bacterial communities

**PO31\_A** - LUNA M. VAN DER LOOS, - Highly divergent Picorna- and CRESS-like viruses associated with bleached thalli of the green seaweed *Ulva* 

**PO32\_A** - XIANYING WEN - Immune resistance in the red alga *Dasysiphonia japonica* against the oomycete pathogen *Olpidiopsis heterosiphoniae* 

### SYMPOSIUM 11 "CHANGING DISTRIBUTION PATTERNS AND NEW ECOSYSTEMS"

**PO33\_A** - ANA CAR - Development of benthic diatoms on natural and artificial substrates in marine lake (South Adriatic Sea)

**PO34\_A** - SUSANA COELHO - Repeated evolution of asexuality in the brown alga *Scytosiphon* 

**PO35\_A** - IRIS DUPČIĆ RADIĆ - Influence of physico-chemical parameters on colonization of bacteria and diatoms in a marine lake in southern Adriatic (NE Mediterranean)

**PO36\_A** - SOFIA HERNÁNDEZ-CHAN - Predicting thermal tolerance limits of marine forests' seaweed species under a changing climate

**PO37\_A** - RAJAA KHOLSSI - Evaluation of short-term copper toxicity in *Synechococcus sp, Chaetoceros gracilis* and *Pleurochrisys roscoffensis* under changes of temperature and salinity

**PO38\_A** - MARIA JOSE LAGUNES - Assumptions and model structure for the growth and survival of the holopelagic brown macroalga *Sargassum* spp. based on DEB (Dynamic Energy Budget) theory

**PO39\_A** - MARINE LASBLEIZ - Characterisation of opportunistic bloom-forming macroalgae other than *Ulva* sp. in green tide bays of Brittany

**PO40\_A** - DETLEV LOHSE - High Frequency Monitoring of Phytoplankton Distribution with Special Reference to Cyanobacteria in a Freshwater Lake

**PO41\_A** - LJILJANA IVEŠA - Adaptation to temperature extremes could improve marine forest restoration success. A case study for a unique, isolated *Gongolaria barbata* population in the northern Adriatic Sea

**PO42\_A** - FLORIAN WEINBERGER - Non-native hosts of an invasive seaweed holobiont have more stable microbial communities compared to native hosts in response to thermal stress

**PO43\_A** - MAYALEN ZUBIA - Harvesting *Turbinaria ornata* for coral reef restoration actions in French Polynesia

### SYMPOSIUM 13 "Taxonomy and Systematics"

**PO44\_A** - MARIA L. PARAGES - *De novo* transcriptome characterization of *Ulva lacinulata* under *in situ* emersion/immersion cyclic conditions

### SYMPOSIUM 15 "Algae as producers of valuable compounds"

PO45\_A - BERK AKSOY - Protein potential of a Portuguese Spirulina strain

 $PO46\_A$  - MARIANA ASSUNÇÃO - Unlocking a nature treasure-chest : the lipid profile of Eustigmatophyceae

**PO47\_A** - TERESA AYMERICH - High hydrostatic pressure assisted antimicrobial extraction from fucoid *Ericaria selaginoides* 

**PO48\_A** - ESTHER BERRENDERO GÓMEZ - Screening of cyanobacteria for their ability to produce bioproducts

 $PO49\_A$  - ADRIANA BLASZCZYK - Diatom frustules – tools for vitamin encapsulation and release

PO50\_A - MANON CHOULOT - A sustainable approach for the seaweed use in agriculture

**PO51\_A** - SOLÈNE CONNAN - Mycosporine-like amino-acids from macroalgae and cyanobacteria sampled in French Polynesia

**PO52\_A** - PRISCILA CORRÊA - Experimental design to optimize Bold's Basal Medium to increase biomass and pigments production in three microalgae species

**PO53\_A** - KAREL DAVALLE-CORRE - The brown macro-alga *Bifurcaria bifurcata*: an opportunity for the control of the parasitic nematode *Heligmosomoides polygyrus bakeri* in animal production

**PO54\_A** - NADEESHANI DEHEL GAMAGE - Novel 'Biofilm PSBR' (Porous substrate photobioreactor) for benthic diatom cultivation

**PO55\_A** - WENDY DELPONT - Seaweed-derived carbohydrates as a barley bio elicitor against infection with the fungus *Ramularia collo*-cygni

PO56\_A - JOANNE FIELD - Algal inks, the future sustainable alternative for labelling

**PO57\_A** - CAMBERRA GAUYAT - Biochemical comparison between two populations of *Sargassum muticum*, introduced in the Pacific coast of Mexico and Atlantic coast of Brittany (France)

**PO58\_A** - PAULINE HAMON-GIRAUD - Reconstruction and enrichment of the genome-scale metabolic network of the Fucale *Ascophyllum nodosum* and its microbiota with bioinformatics methods

PO59\_A - DAVID M. HARTNELL - Microalgal Ecotoxicological Testing: A Lipidomic Approach

**PO60\_A** - ANNA ÞÓRA HRÓLFSDÓTTIR - Physicochemical- and bioactive properties of acid preserved *Alaria esculenta* and *Saccharina latissima* during storage

**PO61\_A** - HSU YEE HTET- The impact of enhancing tolerance to Rose Bengal on growth, photosynthesis and lipid metabolism in *Nannochloropsis oceanica* under suboptimal conditions

**PO62\_A** - ANNA ISAIA - Physiological responses of a tropical microalgae, *Tetraselmis sp.*, to UV-A stress in continuous culture: biomass, antioxidant activity, and photosynthetic efficiency

**PO63\_A** - CHUNZHI JIN - Bioactivity screening of indigenous microalgae from freshwater for cosmeceutical application

**PO64\_A** - SAMY KEMEL - Hydrocarbon continuous production and non-destructive extraction from the microalga *Botryococcus braunii* 

**PO65\_A** - NOLWENN KERGOSIEN - Differential growth of gut, skin and soil bacteria induced by compounds isolated from brown macroalgal species collected on Atlantic coasts, with potential application for industry

**PO66\_A** - HEE-SIK KIM - Microalgae-Based Integrated Biorefinery for Simultaneous Production of Commercially Valuable Carotenoids and Plastic Composites

**PO67\_A** - SANG MIN KIM - The effect of different light intensities and nitrogen concentrations on the interdependence of fucoxanthin, chlorophyll a/c-binding protein and fucoxanthin biosynthesis in *Phaeodactylum tricornutum* 

**PO68\_A** - FANNY LALEGERIE - BlueBio MINERVA: Exploring the potential of Irish macroalgae for sustainable valorisation with focus on *A*. nodosum and *S*. *latissima* 

**PO69\_A** - FANNY LALEGERIE - BlueBio MINERVA: Unlocking the potential of macroalgae for a sustainable valorisation in BlueBioeconomy, using new processes and a biorefinery approach

**PO70\_A** - FLORA LAM KIM - Spatial variability of phlorotannins extracted from *Hormosira banksii* along a longitudinal gradient in Victoria (Australia)

**PO71\_A** - HYUNG-GWAN LEE - Enhancement of carotenogenesis and neutral lipid production by heterologouse overexpression of orange protein in *Chlamydomnas* 

**PO72\_A** - MATHILDE LESGOURGUES - Characterization of bioactive oligosaccharides isolated from the red algae *Solieria chordalis* 

**PO73\_A** - SHOWE-MEI LIN - Sporelings and growth of the marine red alga, *Gelidium elegans* (Rhodophyta), from Northeast Taiwan

**PO74\_A** - DAVID MORGADO - Real time, non-invasive monitoring of microalgae biofilm-based cultivation systems using reflectance spectroscopy

**PO75\_A** - MOUSTAPHA NOUR IBRAHIM - Potential of Djiboutian abundant macroalgae as food and feed ingredients

**PO76\_A** - NUNO NUNES - Assessment of the biochemical parameters of *Arthrospira platensis* cultivated semi-continuously using three nutrient replenishment media for industrial production

**PO77\_A** - FEDERICO ORTENZI - Microalgal-based systems for sustainable wastewater upcycling, bioprocess optimization by mechanical stress

**PO78\_A** - SEMA SIRIN - Multipurpose use of microalgae for sustainable agriculture as bioremediator of hydroponic greenhouse effluent and bioagrichemicals

**PO79\_A** - SOFIIA TRETIAK - Optimized extraction and separation processes for seaweedderived polysaccharides and polyphenols for industrial applications

**PO80\_A** - ASHER WISHKERMAN - Microalgae cultivation system based on additive manufacturing technology

**PO81\_A** - YIRU ZHAO - Assessment of methane and hydrogen production from the brown seaweed *Sargassum* 

### Tuesday 22<sup>nd</sup> August – 3:00pm to 4:25pm

SYMPOSIUM 02 "Biodiversity assessment and algal distribution in space and time"

PO1\_B - ALGAETRAITS EDITORS - AlgaeTraits.org: a trait database for seaweed species

**PO2\_B** - JASNA ARAPOV - The occurence of new *Pseudo-nitzschia* speceis: *P. brasiliana*, *P. hasleana* and *P. linea* in the Adriatic Sea

**PO3\_B** - BETH BEAR - *Akashiwo sanguinea* – 10 years in Wales

**PO4\_B** - FRANCESCO BELLAMOLI - Chlorophyll-*f*-producing cyanobacterial communities in selected karst caves of Northern Italy

**PO5\_B** - TINA BONAČIĆ - Diversity and toxigenic characteristics of cultured *Pseudo-nitzschia* species from East Adriatic Sea

 $PO6\_B - \text{MAMIE SOUADOU DIOP} - DNA \text{-sequencing-based assessment of seaweed biodiversity} and distribution along the Senegalese coast$ 

 $PO7_B$  - GUILLAUME DUPONT - Modelling of the effect of kelp harvesting and herbivory on fragmented forests

**PO8\_B** - TRISTAN GOBERT - Arsenic dynamics during stranding and degradation of holopelagic *Sargassum* spp. in the Caribbean region

**PO9\_B** - ANGÉLIQUE GOBET - Evaluation of sequential filtration and centrifugation to capture environmental DNA and survey microbial eukaryotic communities in aquatic environments

 $\label{eq:PO10_B-LANA} PO10\_B - LANA \ GRIŽANČIĆ - A \ metabarcode \ based \ (species) \ inventory \ of the northern \ Adriatic \ phytoplankton$ 

**PO11\_B** - INKA BARTSCH - The SeaStrains Network – developing a European concept for safeguarding seaweed genetic material to promote food security, biosecurity, and conservation of biodiversity

**PO12\_B** - SIMON KLINE - Seasonal dynamics of the protist community in the Oslofjord and Skagerrak in a changing ocean

**PO13\_B** - WIEBE KOOISTRA - Resolving cryptic species complexes in marine protists: haplotype networks meet global DNA metabarcoding datasets

PO14\_B - MANEL LEIRA - Diatoms as epiphytes on crustose red algae

**PO15\_B** - MANEL LEIRA - Microphytobenthos communities living on *Laminaria* forests by DNA metabarcoding

**PO16\_B** - MAUD LEMOINE - PHYTOBS-Network: French National Service of Observation program for Phytoplankton in coastal waters

**PO17\_B** - PETRA LUČIĆ - Genus *Asparagopsis* (Bonnemaisoniales, Rhodophyta) in the Croatian part of the Adriatic Sea

**PO18\_B** - CLIO MARIDAKIS - Species diversity and distribution of the genus *Jania*, at global scale with a European focus

**PO19\_B** - BEATA MESSYASZ - Freshwater ecosystem bioengineer on a small and large scale (*Cladophora*, Chlorophyta)

**PO20\_B** - SIMON OIRY - Seagrass vs. green macroalgae discrimination: from a hyperspectral library to Drone mounted multispectral camera

**PO21\_B** - ANJELINA POTIN - Marginal hotspots of genetic diversity of red gold *Gelidium corneum* along its biogeographical range

**PO22\_B** - BEGOÑA SÁNCHEZ-ASTRÁIN - Experimental demonstration of recovery capacity of *Gelidium corneum* populations based on lab-induced reattached individuals

**PO23\_B** - SOFIA SADOGURSKA - The genus *Cystoseira* s.l. in Israel (the Levantine basin of the Mediterranean Sea)

**PO24\_B** - KATIA SCIUTO - First data on a green coccoid microalga isolated from Lido of Spina (Comacchio, Ferrara, Italy)

**PO25\_B** - KJERSTI SJØTUN - Mapping and DNA barcoding the brown algal diversity within the orders Ectocarpales, Ralfsiales and Sphacelariales in Norway

**PO26\_B** - SANDA SKEJIĆ - From species to community: long-term dynamics of phytoplankton biodiversity across environmental gradients

**PO27\_B** - PETRA SLAVINEC - Diversity and seasonality of coccolithophores in Slovenian coastal waters (Adriatic Sea)

**PO28\_B** - RONNY STEINBERG - Biomass increase and maturation of North Sea kelp forest despite ocean warming

**PO29\_B** - RAÜL TRIAY-PORTELLA - Habitat suitability of rhodolith seabeds in an oceanic archipelago: regional-scale physical and physiological drivers

**PO30\_B** - DANIEL VAULOT - The  $PR^2$  ecosystem: a set of interconnected databases to map the distribution of micro-algal taxa

#### SYMPOSIUM 06 "Algal cell biology and ultrastructure"

**PO31\_B** - FLORENT CHARTON - Global transcriptome of short- and long-term hyposaline acclimation in a marine diatom: links to the non-coding genome

**PO32\_B** - TIMOTHÉE CHAUMIER - PhaeoEpiView: An epigenome browser of the newly assembled genome of the model diatom *Phaeodactylum tricornutum* 

 $PO33\_B - \text{ANTONIN CHEVENIER} - Discovery of red algal carbohydrate sulfotransferases and their implication in cell wall biosynthesis$ 

**PO34\_B** - AYUSHI DALMIA - Activity-based protein profiling (ABPP) of the green microalga *Lobosphaera incisa* for identification of functional serine hydrolases

**PO35\_B** - MATTHEW DOWSON - Building Interactomes in the Cyanobacterium *Synechococcus elongatus* PCC 7942 Using Large-scale Affinity Purification Mass Spectrometry

**PO36\_B** - CHAN YOUNG HONG - Cell repair in *Griffithsia monilis* mediated by ROS and calcium signaling

**PO37\_B** - RAFAL KRELA - Profiling histone post-translational modifications in three green algae species

**PO38\_B** - GONZALO MENDOZA-OCHOA - Studying a B12-regulated promoter in *Phaeodactylum* and *Chlamydomonas* 

 $PO39\_B - SABINA MUSIAŁ - Dissecting the pyrenoid composition of the red alga Porphyridium purpureum$ 

**PO40\_B** - VINCENT NORMANT - Molecular and cellular effects of trivalent and hexavalent chromium in *Chlamydomonas reinhardtii* 

**PO41\_B** - ABIGAIL J. PERRIN - CyanoTag: High-throughput protein tagging in photosynthetic bacteria

PO42\_B - TRANG VUONG - Life of Chlamydomonas reinhardtii in a nature-like environment

**PO43\_B** - MHAMMAD ZARIF - Impacts of the loss of a histone post-translational mark on the physiology of the pennate diatom *Phaeodactylum tricornutum* 

### SYMPOSIUM 08 "Algal photosynthesis, carbon fixation and respiration"

**PO44\_B** - DORIAN A. BAS - Multi-scale temporal variability in MPB biomass and production in diatom- and euglenophyte dominated estuarine biofilms

**PO45\_B** - MAXINE C. CANVIN - Quantifying Rates of Growth, Erosion and Dislodgment of Cultivated Kelp in the UK

**PO46\_B** - JAVIER CREMADES UGARTE - Using different light-emitting diodes (LEDs) in *Ulva ohnoi* (Ulvales, Chlorophyta) cultures. Implications in IMTA-RAS systems

**PO47\_B** - DANY CROTEAU - Peculiarities of diatom ECS allow *in vivo* measurements of the transthylakoidal proton gradient ( $\Delta pH$ )

**PO48\_B** - GWENAËLLE GAIN - Measurements of photosynthesis in mixture reveal the role of nutrients and allelopathy in the competition between dinoflagellates and diatoms

 $PO49_B$  - CATERINA GEROTTO - Impact of sulfur limitation on marine microalgae cell composition and photosynthesis

**PO50\_B** - SÉBASTIEN GUÉRIN - Diel photosynthesis rhythm in diatoms: evidence of endogenous circadian regulation

**PO51\_B** - MICHAL HUBÁČEK - Strong heterologous electron sink outcompetes alternative electron transport and elucidates coordination of electron distribution from PSI

**PO52\_B** - JASON HUPP - Optical properties of an aerated sample cell in an open-flow through steady-state gas exchange system

**PO53\_B** - VINCENT MERIOT - Targeted and non-targeted metabolomics of *Heterocapsa* cf. *bohaiensis* under Nickel and Iron high concentration and its photophysiology

**PO54\_B** - FRANCESCA MARCHETTO - Directed evolution of an extremophilic red microalga *Cyanidioschyzon merolae* leads to stable adaptation to high concentrations of heavy metals

**PO55\_B** - MOHAMED FAAZIL MOHAMED DAHLAN - Effect of Rare Earth Element (gadolinium) exposure on microphytobenthos photophysiology

**PO56\_B** - ARIANNA RIZZO - Benthic diatoms growth in artificial hydrogels to mimic their natural habitat and to study their physiological properties

**PO57\_B** - FLORIAN STAHL - Feasibility study for kelp plantation in the German Bight: habitat availability and light requirements of *Laminaria hyperborea* 

### SYMPOSIUM 10 "Ecology of algal systems"

PO58\_B - SARA BARRIENTOS - Declining recruitment in a potential climate refuge for kelp

**PO59\_B** - ANDREA BILAJAC - Site-specific predictive models for estimating wet biomass of macroalga *Gongolaria barbata* from two sites

**PO60\_B** - AURÉLIE BLANFUNÉ - *Lithophyllum byssoides* rims: the first marine Mediterranean ecosystem threatened of collapse?

 $PO61\_B$  - NDEYE COUMBA BOUSSO - Safety considerations of seaweed in Senegal: heavy metals hazards

**PO62\_B** - NDEYE COUMBA BOUSSO - The unusual massive beaching of *Sargassum* around Dakar Peninsula (Senegal, Africa) in autumn 2022: opportunity or threat

**PO63\_B** - BENJAMIN CHÂTELAIN - Holopelagic *Sargassum* along a degradation gradient: variation in biochemical composition and impact on potential valorization

**PO64\_B** - OCEANNE-AMAYA CIPOLLONI - Temporal fluctuation of metallic and metalloid concentrations in three morphotypes of floating holopelagic *Sargassum* from Caribbean coast (Guadeloupe, French West Indies)

**PO65\_B** - PABLO COBOS - Invasive alga *Rugulopterix okamurae* relies on high resilience to elevated CO<sub>2</sub> and nutrient limitation to expand in the Mediterranean

**PO66\_B** - WENDY DIRUIT - Remote sensing vs. in situ data: a comparative analysis of seaweed cover assessment on rocky shores

**PO67\_B** - ANNEMIEKE DROST - Matter of traits: Combined effects of nutrients and climate change on cyanobacterial bloom toxicity across scales

**PO68\_B** - BENTE EDVARDSEN - Growth preferences of the haptophyte *Chrysochromulina leadbeateri* forming ichthyotoxic blooms in fjords in N Norway

PO69\_B - CRISTINA GALOBART - Impacts of habitat fragmentation on macroalgal forests

**PO70\_B** - EDI GLJUŠĆIĆ - Epiphyte community in two prominent fucalean algae on the western Istrian coast

**PO71\_B** - JULIA GOSTYŃSKA - The mechanism of competition between coexisting aquatic taxa on the example of *C. glomerata* (L) Kütz. and *L. minor* L. and cyanobacteria in the body of water

**PO72\_B** - ANKE KREMP - Sediments as reservoirs of unexpected pelagic diversity: Distribution of the dormancy trait in Baltic phytoplankton communities

**PO73\_B** - CAROLIN PETER - Response of the cold-adapted spring bloom community to increased nitrogen in the Baltic Sea

**PO74\_B** - FERNANDO QUEIROGA - In vitro effects of the harmful benthic dinoflagellates *Ostreopsis* cf. *ovata* and *Prorocentrum hoffmannianum* on immune responses of the farmed oyster *Crassostrea gasar* 

 $PO75_B$  - RAQUEL SÁNCHEZ DE PEDRO - Warming rate shapes the thermal tolerance of freshwater phytoplankton

**PO76\_B** - CARLOS SANGIL - Early steps in the primary succession of seaweed communities at the Tajogaite volcano lava flows (Canary Islands, Spain)

**PO77\_B** - IZABELĖ ŠUIKAITĖ - Ecology and phylogeny of alien *Raphidiopsis raciborskii*, *Chrysosporum bergii*, *Sphaerospermopsis aphanizomenoides* and *Cuspidothrix issatchenkoi* from three hypertrophic Lithuanian lakes

**PO78\_B** - JANA VERDURA - Grazing effects on recruits of Mediterranean canopy-forming macroalgae: implications for conservation actions

### Thursday 24<sup>th</sup> August – 3:00pm to 4:25pm

SYMPOSIUM 03 "Phylogenetics, Phylogenomics and Evolution"

**PO1\_C** - LORAINE BRILLET-GUÉGUEN - The Phaeoexplorer Genome Database: a Multi-Scale Genomic and Transcriptomic Data Resource for the Brown Algae

**PO2\_C** - LORAINE BRILLET-GUÉGUEN - The Rhodoexplorer Genome Database: a Multi-Scale Genomic and Transcriptomic Data Resource for the Red Algae

 $\ensuremath{\text{PO3}\_C}$  - AHMED DEBIT - LncPlankton: an extensive collection of lncRNAs from marine plankton

**PO4\_C** - CÉLINE DUC - The sophisticated network of histone chaperones is conserved in the pennate diatom *Phaeodactylum tricornutum* 

**PO5\_C** - MATTHEW FREILER - Novel Plastome Assembly and Annotation of Florideophyceae

**PO6\_C** - MARIE-LAURE GUILLEMIN - Nuclear and mitochondrial genetic clines in a red alga presenting multiple transition zones

**PO7\_C** - CÉCILE HERVÉ - Polysaccharides from the extracellular matrix in brown algae: origins and evolutive histories

**PO8\_C** - CAROLINE JUERY - What make *Phaeocystis* species putatively good symbiont for photosymbiosis?

**PO9\_C** - KIRSTEN M. MÜLLER - Rates of evolution in chloroplast genes within the only freshwater species of the Bangiales, *Bangia atropurpurea* 

PO10\_C - ELLYN ROUSSELOT - A comprehensive study of histone proteins in brown algae

**PO11\_C** - LEILA TIRICHINE - Genome wide assessment of genetic diversity and transcript variations in 17 accessions of the model diatom *Phaeodactylum tricornutum* 

### SYMPOSIUM 05 "New insights into the mechanisms and regulation of life cycles in algae"

**PO12\_C** - CHRISTINA ARVANITIDOU - Role of the genome-wide distribution of the epigenetic marks H3K27ME3 and H3K4ME3 in the microalgae *Ostreococcus tauri* 

**PO13\_C** - MAXIM BOKOV - Formation and dynamics of the guanine crystals regarding the light adaptation of dinoflagellate *Amphidinium carterae* 

**PO14\_C** - FERNANDO BOLÍVAR - Biological and sustainable methods to control biofilms in the ornamental fountains of the Alhambra and the Generalife

**PO15\_C** - FREDERIC CHAUX - Photosynthetic performances and photoprotection strategies in life cycle stages of the coccolithophore *Calcidiscus leptoporus* 

**PO16\_C** - YU JUNG CHOI - Developmental signaling during spore division and morphological differentiation in the red algae *Bostrychia moritziana* 

PO17\_C - OLIVIER GODFROY - Basal Cell Fate Determination in Ectocarpus

**PO18\_C** - FRANZ GOECKE - Changes of nuclear DNA content estimates during the life cycle stages of sugar kelp, *Saccharina latissima* 

**PO19\_C** - AGNÈS GROISILLIER - Exploring the role of epigenetic mechanisms in the salinity stress response of the model diatom *Phaeodactylum tricornutum* 

PO20\_C - KAWTHER RAHMA HABCHI - Development of sexual type PCR markers for Ulva spp.

**PO21\_C** - SOO YEON KIM - Molecular mechanisms of spore movement and substrate attachment of red alga *Bangia* sp.

**PO22\_C** - NADINE RIJSDIJK - Development of gene probes to track sexual reproduction events in diatoms

**PO23\_C** - SAMUEL SAINZ-VILLEGAS - Effects of temperature and irradiance on the vegetative propagation processes in gametophytes of *Asparagopsis armata* (Florideophyceae, Rhodophyta) mediated by hook-like structures

**PO24\_C** - MAGDALENA STRUGAŁA - Whether and how do polyphenols affect epiphytic diatom biomass?

**PO25\_C** - ELIANE E. ZAKKA - Phenotypic plasticity in early development of *Dictyota* dichotoma

#### SYMPOSIUM 07 "Genomics technologies in algae"

**PO26\_C** - CAMILLE BEAULIER - Characterization of a uranium-tolerant microalga of the genus *Coelastrella* with high potential for the remediation of metalpolluted waters

**PO27\_C** - HONG II CHOI - Mutation Frequency of *Chlamydomonas reinhardtii* Using CRISPR-Cas9 Ribonucleoprotein Is Tripled by Employing *Agrobacterium* Originated Nuclear Localization Signal

**PO28\_C** - LINDA KEIL - An individual database created from genome and transcriptome data allows for proteomic analysis of *Dunaliella* in response to hyperosmotic conditions

**PO29\_C** - YONGTAE KIM - Genetic tool kits in *Pyropia yezoensis* against biotic and abiotic stresses

**PO30\_C** - GUILLAUME MAREC - Identifying novel marine microalgal enzymes for industrial applications using omics approaches

**PO31\_C** - VICTOR MURISON - Towards the clarification of diatom lipid catabolism: an *in silico* genome and transcriptome mining for *Phaeodactylum tricornutum* lipases

**PO32\_C** - OLIVIA J. WYNN - The effect of ocean warming and CO<sub>2</sub> enrichment on *Ecklonia radiata*: investigating molecular responses

#### SYMPOSIUM 09 "The global carbon-cycle"

PO33\_C - MAEVA GESSON - New experimental setup to investigate marine snow fragmentation

**PO34\_C** - ZRINKA LJUBEŠIĆ - Net primary production facilitated by thermohaline forcings in a stratifed oligotrophic system

**PO35\_C** - YVONNE NEMCOVA - *Halimeda tuna* in the Adriatic – carbonate production on the microscale of individual segments along the latitudinal and the depth gradients

**PO36\_C** - YOICHI SATO - To improve the accuracy of carbon fixation by macroalgae – Effect of the morphology on carbon and nitrogen content

**PO37\_C** - FLORIAN WEINBERGER - Seaweeds, currents and sedimentation: a hydrodynamic prediction model

#### SYMPOSIUM 12 "Polar algae in a changing environment"

**PO38\_C** - EMILY L.M. BROADWELL - Adaptation versus plastic responses to environmental controls in snow and glacier algal blooms

**PO39\_C** - OZAN CIFTCI - A draft genome of the snow algae *Sphaerocystis sp.* 

**PO40\_C** - LUISA DÜSEDAU - Intertidal macroalgal communities in the European Arctic

 $PO41\_C-RAQUEL\ CARMONA$  - Warming modifies the seasonal photophysiology and productivity of Arctic macroalgae

**PO42\_C** - LINDA NEDBALOVÁ - Low-temperature outdoor cultivation of polar microalgae: a comparison of tests in central Europe and Svalbard

**PO44\_C** - ELISA K. PETER - Metabolic characterization of glacier ice algae dominated microbial communities blooming on the Greenland Ice Sheet

**PO45\_C** - PAVEL PŘIBYL - Biological soil crust microalgae manipulation experiments in the High Arctic

**PO46\_C** - BJÖRN ROST - The physiological response of the Arctic haptophyte *Phaeocystis pouchetii* to marine heatwaves

**PO47\_C** - KAROLINE SAUBREKKA - Diversity and distribution of Arctic protists in pelagic and sympagic habitats in the northern Barents Sea as revealed by metabarcoding

#### SYMPOSIUM 14 "Algal culture collections: their increasing societal relevance and role as gene repositories"

PO48\_C - CINTIA IHA - Algal bioprospecting using Machine Learning on Omics data

PO49\_C - SABINA MARKS - CCAC - Central Collection of Algal Cultures

**PO50\_C** - ALISHA NELLY - The use of Ulva as a final stage nutrient removal from factory effluent water

**PO51\_C** - CECILIA RAD-MENÉNDEZ - The Culture Collection of Algae and Protozoa (CCAP): preserving diversity for algal research and biotechnology

**PO52\_C** - ROS WATSON - Algae biobanking: cryopreservation at the Australian National Algae Culture Collection

#### SYMPOSIUM 16 "Advances in algal production processes"

**PO53\_C** - ALBERTA DI CAVE - Valorization of Spirulina's biomass as biostimulant in agriculture: development of a green product to improve sustainability and circular economy

**PO54\_C** - ANDREA FANESI - Bubbling for light: fighting microalgae biofilms development in photobioreactors

**PO55\_C** - ROWON KIM - The Cellular mechanism of chlorosis in *Pyropia yezoensis* and the development of resistant strains

**PO56\_C** - ALEXANDR A. LUKYANOV - Bio-inspired materials for nutrient biocapture and antibiotics biodegradation in wastewater: microalgal cells immobilized on chitosan-based carriers

**PO57\_C** - MELPOMENI SOFIA MENTE - *Chlorella vulgaris* cultivation in low-cost photobioreactors

**PO58\_C** - LORENZO MOLLO - A microalgal consortium for the remediation of ammonium-rich wastewaters: species proportion variability and physiological analysis

**PO59\_C** - R.A. NARAYANAN - A consistent and reliable system for studying eucheumatoid biology

**PO60\_C** - QUYEN NHAM - Reduced night biomass loss in large-scale mixotrophic algal cultivation

SYMPOSIUM 17 "Public-Private partnerships in algal research"

**PO61\_C** - ERIK-JAN MALTA - Stimulating algae cultivation in Spain: tools developed by the INNOVALGA project

#### SYMPOSIUM 18 "Algae and the general public" + SYMPOSIUM 19 "Algae, education and career pathways"

**PO62\_C** - JULIET BRODIE - Studying marine macrophytes through citizen science. The case of the Big Seaweed Search Project-Mexico

PO63\_C - ANA YEBRA - Colorimetric analysis of algal pigments from the Alboran Sea

PO64\_C - LORENA CUBEROS - PHYCO-ART. In search of the lost technique

**PO65\_C** - JOHAN DECELLE - PlanktoQuest: a virtual reality animation to explore inside phytoplankton

**PO66\_C** - MAIKE LORENZ - Identification of phytoplankton using artificial intelligence: a case study on freshwater bioindicators

 $PO67\_C$  - CAROLINA PENA-MARTÍN - Littoral monitoring for an experience of teaching and research

**PO68\_C** - ABIGAIL J. PERRIN - Publications & Public Actions: How can Scientists respond to the Climate and Ecological Emergency?

**PO69\_C** - LOÏC TEN-HAGE - Twenty-Year Study of Algal Taxonomic Diversity in the Intertidal Zone of Hœdic Island: A Pedagogical Program for Life Sciences Students

**PO70\_C** - ELZBIETA WILK-WOŹNIAK - When society meets science - results of the survey on cyanobacterial blooms

SYMPOSIUM 20 "Coastal and freshwater systems under human pressure: status assessment, management and conservation"

PO71\_C - SALMA ABOUSSALAM - The Moroccan Mediterranean Sea medelled by Ecopath model

**PO72\_C** - MARÍA ALTAMIRANO - Development of disinfection protocols for fishing gear to minimize the impact of invasive seaweed *Rugulopteryx okamurae* (Dictyotales, Ochrophyta)

**PO73\_C** - THOMAS BUREL - Seaweeds in ports along the Channel-Atlantic Coast of France: factors influencing species assemblages and distribution

 $PO74\_C$  - DANIELA GABRIEL - Alien Invasions: Quantifying the invasiveness risk of macroalgae in the Azores

**PO75\_C** - MARJAN JANBEN - Effect of mobile bottom trawling on microphytobenthic communities in the German Baltic Sea

**PO76\_C** - ILIANA NIKOLOPOULOU - Phytoplankton and Ecological status of natural lakes in Greece

**PO77\_C** - OCEANNE-AMAYA CIPOLLONI - Reduced transfers of metals and metalloids contaminants from pelagic *Sargassum* spp. accumulated in artificial floating barrier device

**PO78\_C** - EMILIO SOLER ONÍS - Canarian Harmful Algae Observatory: new trends in the study and monitoring of HABs in the Canary Islands, Spain

**Book of Abstracts** 

### PLENARY

# Sexual and asexual reproduction in rhodophytes: evolution, genetic diversity and adaptive strategy

#### Destombe, C.

International Research Laboratory (IRL) 3614, Evolutionary Biology and Ecology of Algae, Centre National de la Recherche Scientifique (CNRS), Sorbonne Université (SU), Pontificia Universidad Católica de Chile, Universidad Austral de Chile, Station Biologique de Roscoff CS 90074, Place Georges Teissier, 29688 Roscoff, France.

Christophe.destombe@sb-roscoff.fr

Genetic diversity within populations depends mainly on how individuals reproduce. Thus, the mode of reproduction shapes the evolutionary trajectories of species. The diversity of reproductive strategies in rhodophytes presents exciting opportunities for theoretical and empirical research. Their haplodiplobiontic sexual cycle is generally characterized by a spatio-temporal uncoupling of meiosis and fertilization with alternating haploid (gametophytes) and diploid individuals (tetrasporophytes). This life cycle has the particularity to have non-flagellated male gametes (spermatia) and a phase of zygote amplification by the female (cystocarp). The absence of a flagellum has been considered a reproductive disadvantage since fertilisation depends essentially on gamete transport by water movements and the cystocarp has been proposed to mitigate inefficiency of fertilisation by non-motile sperm. Despite the abundance of details on the sexual process of red algae, obtained primarily in the laboratory, little information is available on the reproductive ecology and estimation of mating system in the field.

Gametophytes are generally dioecious, but bisexual individuals have been observed in a large number of species; it is unclear how often such life cycle variation occur in natural populations and what the eco-evolutionary consequences are? In an attempt to better understand these phenomena related to sexual reproduction, we studied different populations of *Gracilaria* spp. using demographic monitorings, paternity analyses, controlled crosses, population biology and population genetics studies. Our results challenge several paradigms related to sperm dispersal in rhodophytes. Furthermore, the combination of life history and genetic data allows for the detection of species complexes that include both sexual and asexual lineages.

### **SYMPOSIUM 01 "Taxonomy and Systematics"**

### **KEYNOTE**

# One Step Forward and Two Steps: An Overview of the Taxonomy, Systematics and Classification of Diatoms in the last 2 Decades

#### Kociolek, J.P.

Museum of Natural History and Department of Ecology and Evolutionary Biology, University of Colorado, Boulder, CO, 80309 USA

Patrick.kociolek@colorado.edu

Over the past 20 years there has been an explosion of new information (discoveries of new genera and species, molecular data, formal analyses of phylogenetic relationships and biogeographic patterns), resulting in new understandings of taxon relationships and implications of those relationships related to the distributions over space and time and on the classification of diatoms. These results are reviewed and examples given of the new insights gained through this dynamic period of discovery and innovation. Three bases for the current classification of diatoms, namely convenience, conservative characters and complexity are reviewed and specific cases to refute these approaches are described.

While these advances are helping to move forward a phylogenetic approach to the study and classification of diatoms, the discipline is challenged by an apparent reticence to describing some groups, especially in the context of phylogenies derived from molecular data. The implications of this "New Conservatism" in diatom taxonomy and classification is discussed. While in other groups of algae the search for a natural classification is creating both controversy and new insights, for the diatoms significant hurdles still exist.

Finally, as discoveries highlight the many relationships diatoms have with other community members in the "Phycoverse", the relationship between Cyanobacteria and the diatom lineage Rhopalodiales is explored. The origin and early evolution of a new N-fixing organelle is detailed. Higher rates of extinction in the Rhopalodiales may counter our current views on how endosymbiotic events may be less rosy than current explanations of them may suggest.

### ORALS

# The resurrected genus *Hydrocytium* expands the phylogenetic, morphological, and genomic diversity of the poorly known green algal order Chaetopeltidales

Barcytė, D. & Eliáš, M.

Department of Biology and Ecology, Faculty of Science, University of Ostrava, Czech Republic dovile.barcyte@gmail.com

Chaetopeltidales is a small understudied order in the OCC clade of the green algal class Chlorophyceae, slowly expanding by occasional discoveries of novel algae. In this study, we demonstrate that hitherto unrecognized chaetopeltidaleans exist also among previously described but neglected and misclassified species.

#### MONDAY 21<sup>st</sup> August 2023 Room « Tali ebrel »

The strain *Characium acuminatum* SAG 40.91, with previous preliminary evidence for its affinities to the OCC clade, was investigated with light and electron microscopy to characterize its morphology and ultrastructure. Sequence assemblies of the organellar and nuclear genomes were obtained and utilized in bioinformatic and phylogenetic analyses to address the phylogenetic position of the alga and its salient genomic features.

The characterization of the strain SAG 40.91 and a critical literature review led us to reinstate the forgotten genus *Hydrocytium* A. Braun 1855, with SAG 40.91 representing its type species *Hydrocytium acuminatum*. Independent molecular markers converged on placing *H. acuminatum* as a deeply diverged lineage of the order Chaetopeltidales, formalized as the new family Hydrocytiaceae. Both chloroplast and mitochondrial genomes shared characteristics with other members of Chaetopeltidales and were bloated by repetitive sequences. Notably, independently on Volvocales the mitochondrial *cox2a* gene was transferred into the nuclear genome in the *H. acuminatum* lineage. The nuclear genome data from *H. acuminatum*, as well as from another chaetopeltidalean reported by others, revealed endogenized viral sequences corresponding to novel members of the phylum *Nucleocytoviricota*.

To conclude, the resurrected genus *Hydrocytium* expands the known diversity of chaetopeltidalean algae and provides the first glimpse into their virosphere.

# New insights into the phylogenetic relationship of the *Merismopedia* and *Microcrocis* (Cyanobacteria) genera

Pokorný, J.<sup>1</sup>, Kaštovský, J.<sup>1</sup> & Štenclová, L.<sup>1,2</sup>

<sup>1</sup>University of South Bohemia, Faculty of Science, Branišovská 1760, České Budějovice, 370 05 Czechia <sup>2</sup>Institute of Hydrobiology, Biology Centre CAS, Na Sádkách 702/7, České Budějovice, 370 05 Czechia pokorj13@prf.jcu.cz

The genera Merismopedia and Microcrocis have been considered as distinct members of the family Merismopediaceae (Cyanobacteria), both possessing strong morphological resemblance, mainly the typical flat one-layered colonies. Based on scarce molecular evidence, recent studies presumed that Merismopedia, characteristic by its spherical cell shape, is rather a polyphyletic genus, moreover, with unclear relationships to its cylindercelled twin Microcrocis. Our research partly clarified the phylogeny and taxonomy of these elusive cyanobacterial taxa. The complex methodology included strain isolation or single colony sequencing, provided by direct two-step PCR or PCR with an intermediate step of multiple displacement amplification and frequent cloning, morphological (LM) and ultrastructural (TEM) observation. The crucial step for the revision was arranged since the Merismopedia-like material was obtained from its type locality. Phylogenetic analyses performed on roughly two hundred isolates and strain sequences corresponding to more than ten morphospecies proved even more complicated polyphyly of both genera. Taxonomic changes and new combinations are expected. Substantial part of the variability including the epitype of *Microcrocis geminata*, the type species of the genus, and the material from the type locality of the type species of Merismopedia: Merismopedia punctata was progressively placed in Geminocystaceae. This was credibly supported by the determination of evolutionarily unique parallel-arranged thylakoids. Specimens of both genera possess potentially interesting movement newly documented here in ongoing study.

### The importance of genomics in polyphasic identification of novel thermophilic cyanobacteria

Daroch, M.<sup>1</sup>, Tang, J.<sup>2</sup>, Jiang, Y.<sup>1</sup>

<sup>1</sup>School of Environment and Energy, Peking University Shenzhen Graduate School, Shenzhen, 518055, China <sup>2</sup>School of Food and Bioengineering, Chengdu University, Chengdu 610052, Sichuan, China m.daroch@pkusz.edu.cn

Thermophilic cyanobacteria are widely distributed in hot spring ecological niches and are primary photosynthetic producers of geothermal ecosystems, accounting for a large part of those ecosystems' biomass and productivity. Meanwhile, their biotechnological and environmental potential is underutilised, and their taxonomy often requires significant revision.

The taxonomy of cyanobacteria has always been controversial, as two parallel systems of delineation can be applied simultaneously; the bacteriological code based on the ICSP and the botanical code based on the ICN. These issues are further exacerbated by the extremely simple morphology of many unicellular and filamentous strains. As a result, Cyanobacteria are frequently named with umbrella terms such as Synechococcus, Leptolyngbya or Oscillatoria.

One of the ways to address this issue is to apply polyphasic identification, combining morphological characteristics, distinct ecological features, and marker-based taxonomy, usually comprising 16S and ITS markers. However, despite the success of the polyphasic approach in delineating new families, genera and species, there is a massive underutilisation of modern genomic tools in the taxonomic allocation of new and existing strains, and this underutilisation results in misallocations.

Here we describe isolation, genomics-supported polyphasic identification, delineation of two new genera of filamentous thermophilic cyanobacteria: Leptothermofontia and Trichothermofontia and focus on species delineation and reorganisation of strains in other clades with a strong presence of thermophilic cyanobacteria: Thermoleptolyngbya, Leptodesmis, Albertania, and Thermosynechococcus. The study combines the classical polyphasic approach with whole genome sequencing and the application of both global genomic indexes and taxogenomics to guide the more accurate taxonomic allocation of novel cyanobacteria.

#### 200 years of *Microcoleus* – untangling the taxonomy in species continuum

### Skoupý, S., Stanojković, A., Dvořák, P.

Palacký University Olomouc, Faculty of Science, Department of Botany, Šlechtitelů 27, 78371 Olomouc, Czech Republic

\*svatopluk.skoupy01@upol.cz

The global biodiversity of cyanobacteria still remains poorly understood. Cyanobacterial diversity was mainly studied using morphological and ecological data, before genetic data became widely used. Many cyanobacterial species still have only morphological data available, as they were described before the advent of molecular methods and are thus in dire need of revisions. Gene flow, horizontal gene transfer and high levels of cryptic diversity all complicate species delimitation in cyanobacteria. We used a combination of phylogenomic, population genomic and population level morphological data to delimit the species boundaries. We gathered whole genome sequences and morphological data of 200 strains of Microcoleus obtained from different environments all around the globe. We also obtained whole genome sequences of 8 herbarium specimens of *Microcoleus*, including the type specimen of *Microcoleus* genus. *Microcoleus* genomes in our dataset could be differentiated into 13 putative species, forming a speciation continuum. The morphology was relatively stable within the putative species but varied highly between species. Some species could be identified by distinct apomorphies or by a combination of morphological traits, while others are morphologically cryptic and could not be differentiated without the use of molecular data. We were able to match 6 species to a previously described unrevised species, including 2 species with available herbarium specimens, while 7 species are likely new species. This approach combining population genomic and morphological data with herbarium genomes allows to bridge the centuries of cyanobacterial taxonomy and peek into the true biodiversity of cyanobacteria.

# Is structural colour in the red algal genus *Chondria* (Rhodomelaceae, Chondriae) taxonomically significant?

Arnould-Pétré, M.<sup>1,2</sup>, Le Gall, L.<sup>3</sup> & Brodie, J.<sup>1</sup>

 <sup>1</sup>Science, The Natural History Museum, Cromwell Road, London, SW7 5BD, United Kingdom
 <sup>2</sup>Institut des Substances et Organismes de la Mer (ISOMer), UR 2160, Nantes Université, 44000 Nantes, France
 <sup>3</sup>Institut Systématique Evolution Biodiversité (ISYEB), Muséum National d'Histoire Naturelle, CNRS, Sorbonne Université, EPHE, CP 39, 75005, Paris, France
 m.arnould-petre@nhm.ac.uk

Some species in the red algal genus *Chondria* (Ceramiales, Rhodomelaceae) have structural colour, the phenomenon created by nano-structures of a material splitting up light into the colours of the spectrum and causing iridescence. There are four species of *Chondria* recorded in Britain: *Chondria coerulescens* and *C. scintillans*, which are well-known for their strikingly iridescent fronds, *C. capillaris*, which has recently been seen with iridescent reproductive structures, and *C. dasyphylla* which has no structural colour. In order to see whether there is an evolutionary signal between those species with structural colour and those without, it is necessary to confirm their identity and determine phylogenetic relationships within *Chondria*. Systematic studies on the Chondriae tribe show the need for taxonomic clarification. Molecular phylogenies inferred from COI-5P and *rbcL* gene regions were constructed to map the structurally coloured species and understand where samples collected from Britain relate to those from other regions of the world. The resulting trees show that *C. scintillans* consists of more than one species and structural colour in *Chondria* appears to be spread throughout the genus but is not monophyletic. This has led to additional morphological analyses to complement the results. These results support previous studies pointing to the need for a revision of the Chondriae.

# Assessing the molecular diversity of *Sargassum* (Phaeophyceae) in the NE Atlantic Ocean

Álvarez-Canali, D.<sup>1</sup>, Sangil, C.<sup>1</sup>, Sansón, M.<sup>1</sup> & Tronholm, A.<sup>1,2</sup>

<sup>1</sup>BOTMAR-ULL, University of La Laguna, Canary Islands, Spain <sup>2</sup>Dept. Biological and Environmental Sciences, University of Gothenburg, Sweden dalvarec@ull.edu.es

*Sargassum* plays a key role as habitat-forming species in benthic and pelagic ecosystems in tropical and subtropical regions, and has recently become a global environmental issue due to the societal impact of its massive blooms. With about 1000 taxa described and ca. 350 taxonomically accepted species, *Sargassum* represents almost one-sixth of Phaeophyceae and a prime example of a troublesome

#### MONDAY 21<sup>st</sup> August 2023 Room « Tali ebrel »

taxonomy. Species identification has traditionally been hampered by the phenological plasticity exhibited in response to environmental conditions and life history. The advent of DNA phylogenies has challenged the taxa circumscription of this genus and have proven critical in delimiting the species. However, the molecular diversity of the Atlantic species of *Sargassum* remains poorly understood, and recently a few studies have shown a low genetic diversity between the species in the NW Atlantic. Here, we aim to assess the molecular diversity of *Sargassum* in the NE Atlantic Ocean using a multigene approach, combining markers commonly used in delineating species of this genus (*rbc*LS, ITS2, *cox3*, *mtsp*) and exploring new markers (*cox2*, *nad6*, *psb*C, *clp*C, *atp*B) to determine the phylogenetic relationships between the different morphospecies. Both single-gene and multigene phylogenies confirmed the low genetic variability and did not support distinct species in *Sargassum* in the Atlantic benthic species, probably due to a recent colonization and radiation of this basin. These findings contrast with the morphological and ecological diversity of *Sargassum* in the Atlantic, and showcase the need for further analyses, including population genetics and the use of high-throughput DNA technologies.

### **KEYNOTE**

#### The future challenges of seaweed systematics

Leliaert, F.

Meise Botanic Garden, Meise, Belgium frederik.leliaert@meisebotanicgarden.be

With an estimate of 9,600 described extant species, seaweeds are a diverse and taxonomically challenging group of eukaryotes. Seaweed systematics has come a long way, and represents a vast body of work that has accumulated over the past two and a half centuries. Advances in seaweed taxonomy have often been accompanied by technological or analytical progress, including improved microscopy and culture techniques. Unquestionably, the main revolution in recent decades has come with the application of molecular data, which has profoundly reshaped our understanding of algal diversity and relationships. However, this progress has not been uniform geographically. New methods have also created taxonomic challenges, such as how to deal with cryptic diversity, non-monophyly, and old taxon names. The field of seaweed systematics is transitioning into the genomic era, which will provide further insights into diversity and classification. A second ongoing revolution comes with digital access to collections. Herbarium specimens have long been the cornerstone of taxonomic research. Their digitization is increasing access and is transforming them into new dynamic digital research tools. Digital collections likely will be targeted by machine learning for automated species discovery and delimitation. Together with other digital sources such as literature and DNA sequences, Linked Open Data will further revolutionize the way taxonomic data is managed, shared, and integrated.

### ORALS

# On the Application of European Binomials for Western Atlantic Marine Macroalgae by 19<sup>th</sup> Century Taxonomists

Schneider, C.W.<sup>1</sup> & Saunders, G.W.<sup>2</sup>

<sup>1</sup>Department of Biology. Trinity College, Hartford, Connecticut, USA

<sup>2</sup>Centre for Environmental & Molecular Algal Research, Department of Biology, University of New Brunswick, Fredericton, New Brunswick, Canada cschneid@trincoll.edu

Prior to 1900, macroalgal specimens collected from various sites around the world were collected by or simply sent to European phycologists including C. Agardh, J. Agardh, Harvey, Kützing and Montagne for identification. Just as a comprehensive foundational literature of marine macroalgae was being written, the first specimens collected in the Americas were being identified by these and other workers. In many cases, the familiar morphologies of local species prompted the application of European binomials for their American look-alikes. In the 1850s, Harvey wrote his three-volume first flora for the Americas, *Nereis Boreali-Americana*, focusing on the east and west coasts of the United States. Despite his description of many new species, a great number of the binomials applied to American specimens were the same names as those ascribed to specimens from cold to subtropical European waters. These names remained in the floras of North America for more than a century and a half.

The shift to DNA sequences as a tool to distinguish among species in the mid-1990s enabled researchers to test whether or not species were truly amphi-Atlantic, the same species being found in Europe as well as on the east coast of North America, Bermuda and the Caribbean Sea. We detail many examples of amphi-Atlantic species that we have sequenced in genera from *Champia* to *Wrangelia* that required splitting into different species with more narrow geographic distributions. Those taxa genetically shown to be truly amphi-Atlantic are typically those found in the coldest waters of North America and Europe.

# Proposal for a global taxonomic and nomenclatural framework for eucheumatoids (Rhodophyta)

Lim, P.E.<sup>1</sup>, Poong, S.W.<sup>1</sup>, Tan, J.<sup>2</sup>, Brakel, J.<sup>3,4</sup>, Gachon, C.<sup>5</sup> & Brodie, J.<sup>6</sup>

<sup>1</sup>Institute of Ocean and Earth Sciences, University of Malaya, 50603 Kuala Lumpur, Malaysia

<sup>2</sup>Department of Agricultural and Food Science, Faculty of Science, Universiti Tunku Abdul Rahman, Perak, Malaysia

<sup>3</sup>Helmholtz Institute for Functional Marine Biodiversity at the University of Oldenburg (HIFMB), Ammerländer Heerstrasse 231, 26129 Oldenburg, Germany

<sup>4</sup>Alfred-Wegener-Institute, Helmholtz Center for Polar and Marine Research, Am Handelshafen 12, 27570 Bremerhaven, Germany

<sup>5</sup>Unité Molécules de Communication et Adaptation des Micro-organismes, UMR 7245, Muséum National d'Histoire Naturelle, CNRS, Paris, France

<sup>6</sup>Natural History Museum, Science, London, United Kingdom

phaikeem@um.edu.my

Global demand for eucheumatoids is continuing to increase, with the carrageenan market projected to reach USD 1.18 million by 2028. However, the trend is declining for production among the main carrageenan producers. A concerted effort is therefore needed to reverse this decline to safeguard this industry. Most commercial eucheumatoids used today have been propagated vegetatively for 50 years, resulting in low genetic diversity, leading to reduced growth rates, poor resilience to climate change, and increased susceptibility to pests and diseases. This can be addressed with the development of new cultivars by exploring and harnessing the rich genetic diversity of wild eucheumatoids have been collected from over 10 countries and DNA barcoded. The resulting dataset is of unprecedented breadth and depth for eucheumatoids, uncovering novel genetic diversity within known species, as well as potentially new species and genera. Preliminary data highlighted weaknesses in the current eucheumatoid taxonomic

#### MONDAY 21<sup>st</sup> August 2023 Room « Tali ebrel »

framework, which hinders the correct identification of individuals. This was partly due to the unregulated use of terminologies including cultivar, strain, form, morphotype, as well as an inconsistent naming system in past eucheumatoid studies. This negatively affects crop quality, biobanking and breeding, as well as the monitoring of wild stocks. Via GATEWAY, a Safe Seaweed Coalition project, a multigene eucheumatoid phylogeny coupled with genome skimming data is providing the taxonomic framework foundation with which to develop a standard nomenclature equivalent to the International Code of Nomenclature for Cultivated Plants (ICNCP).

# Diversity of coralline red algae from ecologically relevant habitats in Corsica, western Mediterranean

Peña, V.<sup>1</sup>, Rousseau, F.<sup>2</sup>, Otero-Ferrer, F.<sup>3</sup>, Deter, J.<sup>4</sup>, Ballesta, L.<sup>4</sup> & Le Gall, L.<sup>2</sup>

<sup>1</sup>BioCost Research Group, Department of Biology, University of A Coruña, Spain

<sup>2</sup>Institut de Systématique, Évolution, Biodiversité (ISYEB), Muséum National d'Histoire Naturelle, CNRS, Sorbonne Université, EPHE, Université des Antilles, Paris, France

<sup>3</sup>Grupo en Biodiversidad y Conservación (IU-ECOAQUA), Universidad de Las Palmas de Gran Canaria, Spain.

<sup>4</sup>*Andromède océanologie, Mauguio, France* v.pena@udc.es

v.pena@udc.e

Coralline red algae conform an extremely diverse group with a global distribution. They also function as engineer species creating singular seascapes in the Mediterranean such as the coralligenous and maerl/rhodolith beds. The island of Corsica -western Mediterranean- constitutes an area of high marine biodiversity. More than 500 macroalgal taxa were reported in floristic studies conducted between 1976 and 1989. About 30 species corresponded to coralline algae, including the coralligenous species Pseudolithophyllum cabiochiae (now synonym of Lithophyllum stictiforme) with type locality in Corsica. Between 2019 and 2021, samplings of benthic communities were conducted in various Marine Protected Areas of the island. Coralline algae were collected from the intertidal down to 116 m depth. The species identification was carried out using molecular systematics tools and morpho-anatomical observations. With the high transparency of the water, coralligenous communities are flourishing between 50 and 70 m. Several species of the Lithophyllum stictiforme complex are main components of the two coralligenous morphotypes, the typical morphotype and the morphotype 'coralligéne de plateau'; while the new morphotype known as 'coralligenous atolls' consisting on circular structures located at > 100 m deep are composed of at least one unidentified species of *Lithothamnion*. Maerl/rhodolith beds were also observed from ca. 20 m to > 60 m depth; the genus *Lithothamnion* is found to be the main contributor with at least four species tentatively assigned to L. crispatum, L. minervae, L. valens and L. philippi. To our knowledge, our results contribute to the species richness of biogenic habitats recorded for Corsica.

### Phylogenetic analysis of the red algal tribe Ceramieae reveals multiple morphological homoplasies but defines new genera

Barros-Barreto, M.B.<sup>1</sup>, Jaramillo, M.A.<sup>2</sup>, Hommersand, M.H.<sup>†3</sup>, Ferreira, P.C.G.<sup>4</sup><sup>†</sup> & Maggs, C.A.<sup>5</sup>

<sup>1</sup>Department of Botany, Federal University of Rio de Janeiro, Rua Professor Rodolpho P. Rocco 211, CCS-Bloco A, sala 66, 21941-902, Rio de Janeiro, RJ, Brazil

#### MONDAY 21<sup>st</sup> August 2023 Room « Tali ebrel »

<sup>2</sup>Grupo DIVERSITAS, Facultad de Ciencias Básicas y Aplicadas, Universidad Militar Nueva Granada, km 2 Cajica-Zipaquira, Cajica, Cundinamarca, Colombia USA

<sup>3</sup>Department of Biology, University of North Carolina at Chapel Hill, Chapel Hill, NC 275993280

<sup>4</sup>Department of Medical Biochemistry, Institute of Medical Biosciences, Federal University of Rio de Janeiro, RJ, Brazil

<sup>5</sup>School of Biological Sciences, Queen's University, Belfast BT9 5AJ, N. Ireland beatrizdebb@gmail.com

A defensible evolutionary classification of living organisms is required for biological research in fields as diverse as biotechnology and ecosystem functioning. Despite the traditional importance of morphological characters in classification, DNA sequence data are now more widely used in phylogenetic reconstructions.

The tribe Ceramieae (family Ceramiaceae), a widespread, speciose and morphologically diverse group of red algae, is a good target for exploring issues around molecular-guided taxonomy as morphological similarities may not reflect true phylogenetic relationships. We aimed to elucidate phylogenetic relationships in the tribe to delimit genera and provide a new classification.

We used three molecular markers: the *rbc*L gene, the COI-5P gene and partial large subunit rRNA gene (LSU). Within the Ceramieae, several clades are recognized although the relationships among major clades are not resolved. These clades are here recognized as genera, with new circumscriptions for *Ceramium, Carpoblepharis, Herpochondria, Campylaephora,* and *Celeceras* and the proposal of *Pseudoceramium* Barros-Barreto & Maggs, gen. nov., *Yoneshiguea* Barros-Barreto, M. A. Jaram. & Maggs, gen. nov. and *Stirkia* Barros-Barreto & Maggs, gen. nov.

The Ceramieae show a high degree of morphological homoplasy among species as well as extreme phenotypic plasticity within species. Genera lack unique synapomorphies and multiple morphological characters are required to define most of them. Molecular data are critical for understanding systematics in this group.

# Phylogeny and ecophysiology of golden-brown snow algae (Chrysophyceae) from Svalbard, the European Alps and High Tatras

Procházková, L.<sup>1</sup>, Remias, D.<sup>2</sup>, Nedbalová, L.<sup>1</sup> & Andersen, R.A.<sup>3</sup>

<sup>1</sup>Department of Ecology, Faculty of Science, Charles University, Prague, Czech Republic, <sup>2</sup>Department of Environment & Biodiversity, Paris Lodron University Salzburg, Salzburg Austria, <sup>3</sup>Friday Harbor Laboratories, University of Washington, Friday Harbor, Washington DC, USA lenka.prochazkova@natur.cuni.cz

Melting mountain and polar snowfields harbour diverse microbial communities. We focused on microalgae causing macroscopically visible golden-brown blooms under favourable conditions in the Central Europe (Austria, Slovakia) and the Arctic (Spitsbergen). Field populations and nine newly established algal strains were made of unicells with *Ochromonas* like morphology, however, investigation by molecular means revealed that they are a part of the Hydrurales, and not Ochromonadales. They exhibited a high diversity at the species level in the both geographical regions. The closest molecular relatives were so far uncultured isolates or clones from cold habitats, such as the riverine *Hydrurus foetidus* (Villars) Trevisan and morphologically undescribed algae of environmental sequences from polar snow and polar oligotrophic or mesotrophic lakes. Apparently, the ability to colonize and cause blooms in snow habitats developed several times independently in the Chrysophyta (e.g. this study, *Chromulina chionophilia, Kremastochrysopsis austriaca*). Electron microscopy of the studied flagellates indicated the presence of one long flagellum bearing mastigonemes and a usually

(very) short second flagellum; (abundant) tetrahedral (to hexahedral) zoospores, possibly loosely developed thalli and rarely smooth-walled stomatocysts with a collar were present as well. Photobiology of field samples using fluorometry showed that all high-alpine as well as polar populations were adapted to higher irradiation, thus reflecting ambient light conditions *in situ* (shaded valley *vs.* exposed snow at glaciers; bloom below the snow surface *vs.* reaching the snow surface). In general, the ecological and taxonomical implications between cold lotic and nival chrysophytes exhibit a promising field for polar-alpine research.

# Chemotaxonomy of holopelagic *Sargassum* specimens using molecular networking

Nirma, C.<sup>1</sup>, Stiger-Pouvreau, V.<sup>1</sup>, Petek, S.<sup>1</sup>, Touboul, D.<sup>2,3</sup>, Levasseur M.<sup>2</sup> & Connan, S.<sup>1</sup>

<sup>1</sup>Univ Brest, IRD, CNRS, Ifremer, LEMAR, F-29280 Plouzane, France <sup>2</sup>CNRS, Institut de Chimie des Substances Naturelles (ICSN), UPR 2301, Université Paris-Saclay, Avenue de la Terrasse, 91 198 Gif-sur-Yvette, France <sup>3</sup>Laboratoire de Chimie Moléculaire (LCM), CNRS UMR 9168, École Polytechnique, Institut Polytechnique de Paris, Route de Saclay, CEDEX, 91 128 Palaiseau, France

charlotte.nirma@univ-brest.fr

This work is part of the ANR Sargassum Origins project, which aims to understand the origin of massive holopelagic *Sargassum* development in the tropical North Atlantic Ocean. Three morphotypes belonging to two species have been formally identified in these Atlantic drift rafts: *Sargassum fluitans* III (named S3) and *Sargassum natans* I (S2) and VIII (S1), but difficulties remain in differentiating them molecularly. In order to differentiate these three morphotypes, a chemotaxonomic study was conducted with the aim to identify chemical marker(s) that will allow the three morphotypes to be formally discriminated.

For this purpose, hydroalcoholic extracts of freeze-dried *Sargassum* were analysed by tandem mass spectrometry (RPLC-ESI-HRMS/MS) with Data-Dependent Acquisition (DDA) and the spectra obtained were pre-processed with MZMine 2. DDA allows the fragmentation of precursor ions, in a complex sample, which provides structural information of molecules. Structurally related molecules show similar fragmentations in MS<sup>2</sup>, and the alignment of these spectra allows the construction of molecular networks. The results obtained in this study were represented with t-SNE algorithm of the MetGem software.

The molecular networks obtained highlighted the presence of compounds specific to the S2 and S3 morphotypes, allowing these species to be chemically discriminated. These were particularly galactolipids and sulphoglycolipids, but other compounds are being identified by comparing fragmentation spectra with libraries of known compounds. The use of statistical tests of <sup>1</sup>H NMR spectra of the same extracts confirms the discriminating molecular families identified through the molecular networks.

#### Mastophoraceae diversity within the Mariana Islands

Heagy, MK.<sup>1</sup>, Maneveldt, G.<sup>2</sup> & Schils, T.<sup>1</sup>

<sup>1</sup>University of Guam Marine Laboratory, Mangilao, Guam <sup>2</sup>University of the Western Cape, Cape Town, South Africa heagym@gotritons.uog.edu Guam is a hotspot of crustose coralline algae (CCA) diversity, characterized by a high level of endemism. DNA barcoding efforts have tripled the number of known CCA species for Guam since the last floristic account in 2003. Here we focus on the taxonomic diversity within the family Mastophoraceae (order Corallinales) in the Mariana Archipelago. Collections were made from reefs surrounding Guam, Rota, and Saipan. These islands are all located in the southern Mariana Islands but differ in age, size, and reef structure. Upon collection, Mastophoraceae specimens were photographed, curated, and sequenced (COI-5P and *psb*A). Members of the Mastophoraceae are abundant on most Pacific reefs. As currently described, the Mastophoraceae are the least taxon rich family of the Corallinales with 4 recognized genera and 6 recognized species. The collections from the Mariana Islands, however, suggest a much higher generic and species diversity at local and regional scales. The description of at least eight new Mastophoraceae species is warranted. Differences in species composition between islands in the southern Marianas are apparent. In addition, generic delimitations within the family require systematic revision. Guam's accessible tropical reefs and well-documented marine flora make it an ideal study site for further diversity, ecological, and biogeographical studies of coralline algae.

#### Phylogenomic analyses reveal a new introduced species in Europe: Lophurella stichidiosa comb. nov. (Rhodomelaceae, Rhodophyta)

Díaz-Tapia, P.<sup>1</sup>, Nelson, W.A.<sup>2</sup> & Verbruggen, H.<sup>3</sup>

<sup>1</sup>Instituto Español de Oceanografía, CO-A Coruña, Spain <sup>2</sup>School of Biological Sciences, University of Auckland, New Zealand <sup>3</sup>School of Biosciences, The University of Melbourne, Australia p.diaz@udc.es

Detection of non-native seaweed species is often difficult, particularly for old introductions. Inconspicuous species like small turf-forming algae present extra challenges. Our recent surveys in Australia revealed the occurrence of two species that morphologically resemble the turf-forming *Aphanocladia stichidiosa*, originally described from Naples, Italy in 1955. We aim to resolve the identity of these Australian specimens, to revise their generic placement in the context of the tribe Pterosiphonieae and to interpret the historical biogeography of this group.

One of the Australian species matched *A. stichidiosa* from Europe, showing identical *rbcL* sequences and morphological characters, considerably expanding the known distribution of this species. The second species is new, only known from one site in southeastern Australia. Our phylogenomic analyses based on plastid genomes confidently showed that the two Australian species were related to *Lophurella* instead of the generitype of *Aphanocladia*, so a new combination is proposed. Our phylogenetic analyses showed that the two Australian species are part of a Pterosiphonieae lineage restricted to the southern hemisphere, predominantly Australasia, suggesting that *L. stichidiosa* is native from Australia and may have been introduced in Europe ca. 70 years ago. In Europe, *L stichidiosa* is expanding its distribution, recently into the Azores, and its abundance is increasing in at least some regions. This study showcases the utility of phylogenomic approaches to resolve taxonomic uncertainties and uncover introduced species.

# NGS reveals heteromorphic life history of the red alga Nemastoma dichotomum

Maggs, C.A.<sup>1,2</sup>, Guiry, M.D.G.<sup>1</sup>, Hughey, J.R.<sup>3</sup> & Díaz-Tapia, P.<sup>4</sup>

<sup>1</sup>School of Natural Science, University of Galway, Ireland

<sup>2</sup>School of Biological Sciences, Queen's University Belfast, N. Ireland

<sup>3</sup>Division of Mathematics, Science, and Engineering, Hartnell College, Salinas, California, 93901, United States

<sup>4</sup>Coastal Biology Research Group, Faculty of Sciences and Centre for Advanced Scientific Research (CICA), University of A Coruña, Spain

christine.a.maggs@gmail.com

During sampling of crustose red algae in a fully marine inlet off Kilkieran Bay, Co. Galway, Ireland, in December 1981, a crust was collected in association with tiny erect axes identified tentatively as *Nemastoma* sp. It was bisporangial and bore large gland cells. Spores released in culture from the bisporangia gave rise directly (i.e. apomictically) to bisporangial crusts which themselves became fertile as a short-day response. The culture was maintained for three years without forming erect thalli, and then dried and kept as vouchers. If this crust were shown to be part of the life history of *Nemastoma* sp., it would be the first record of the sporophyte in the field. At around the same time (1982) a separate collection of large erect axes was made in Ireland, at Lough Hyne, Co. Cork, and provisionally identified as *Nemastoma canariense*; it was re-collected in 2001.

New developments in molecular methodology (next-generation sequencing) allowed us to obtain nearly full plastid and mitochondrial genomes from the original cultures. The rbcL sequence of the Kilkieran Bay culture is identical to *N. dichotomum* from the Western Mediterranean (Islas Columbretes, Spain). After four decades, we can now reveal the morphology of the previously unknown sporophyte of *N. dichotomum* and confirm the presence of this species in Ireland for the first time, the first confirmed record outside the Mediterranean. We also obtained sequences from the 2001 Lough Hyne material, which is also *N. dichotomum*, so the report of *N. canariense* from Ireland represents a misidentification.

# Toward an inventory of marine subantarctic algae: investigations in Kerguelen, Crozet, Saint Paul & Amsterdam

Le Gall, L.<sup>1</sup>, Rousseau, F.<sup>1</sup>, Jossart, Q.<sup>2</sup>, Force, A.<sup>2</sup> & Saucède, T.<sup>2</sup>

<sup>1</sup>Institut de Systématique, Évolution, Biodiversité (ISYEB), Muséum National d'Histoire Naturelle, CNRS, Sorbonne Université, EPHE, Université des Antilles, Paris, France <sup>2</sup>Biogéosciences, UMR 6282, CNRS, Université de Bourgogne, Dijon

In the south of the Indian Ocean, the French Southern Territories (Crozet, Kerguelen, SaintPaul and Amsterdam) are particularly well-preserved sanctuaries of biodiversity that contain an exceptional natural heritage. In order to conserve this unique heritage, France created the French Southern Territories National Nature Reserve in 2006, which included the entire land surface of the islands (7,700 km<sup>2</sup>). After a recent extension of the reserve in 2022, the surface of the maritime area now reaches 1 655 000 km<sup>2</sup>, making it the second largest marine protected area on the planet. Those islands ranging from latitude of 38 °S (Amsterdam) to 49 °S (Kerguelen) accommodate a luxuriant flora of seaweeds with impressive biomass of giant kelp (*Macrocystis pyrifera*) surrounding the islands. Nevertheless, the actual diversity of the region is far from being well known and the taxonomy of the subantarctic and Antarctic seaweeds is still challenging. During the summer campaign of 2022, we sampled 24 localities

from the intertidal down to 20 m depth on these four islands. Collected seaweeds were preserved both as voucher herbarium and silica gel samples in the aim to use molecular assisted taxonomy to address the species richness as well as infer the phylogenetic relationships of the taxa.

### SYMPOSIUM 04 "Algae and their intimate partners"

### **KEYNOTE**

# Factors that influence microbiome development and disease in seaweeds

#### Egan, S.

UNSW Sydney, Australia s.egan@unsw.edu.au

Seaweeds host complex and often unique microbial communities (microbiomes), that are thought to provide essential functions for the host, including provision of settlement and morphogenic cues; nutrients; and protection from secondary colonisers and opportunistic pathogens. However, how seaweeds acquire these microbial symbionts, the factors that influence microbiome development and the impact of microbial dysbiosis on seaweed heath remain largely unknown. In this seminar I will present some of our recent findings that support species-dependent processes of symbiont acquisition in seaweeds ranging from selective and non-selective vertical transmission from parents to horizontal transmission from the environment. I will also present work using the green seaweed *Ulva australis* as a model to show how early colonisation by specific bacteria can impact subsequent microbiome development. I will further discuss how anthropogenic stressors may disturb the host microbiome (i.e., cause dysbiosis) resulting in the proliferation of opportunistic pathogens leading to disease. Finally, I will describe recent studies that show mitigation of bleaching disease in the red seaweed *Delisea pulchra*, through the addition of beneficial microorganisms and their possible mode of action.

### ORALS

# The Ascophyllum nodosum holobiont: dynamics of microbial communities over one-year in situ survey

Rousseau, C.<sup>1</sup>, Legeay, E.<sup>2</sup>, Tanguy, G.<sup>2</sup>, Rousvoal, S.<sup>1</sup>, Leblanc, C.<sup>1</sup> & Dittami, S.M.<sup>1</sup>

<sup>1</sup>Sorbonne Université, CNRS, Integrative Biology of Marine Models (LBI2M, UMR 8227), Station Biologique de Roscoff (SBR), 29680 Roscoff, France

<sup>2</sup>Sorbonne Université, CNRS, Genomer Platform, FR2424, Station Biologique de Roscoff (SBR), 29680 Roscoff, France

coralie.rousseau@sb-roscoff.fr

The brown alga *Ascophyllum nodosum* and its microbiota form a dynamic functional entity named holobiont. The microbial partners play a role in seaweed health by producing bioactive compounds crucial for normal morphology and development. *Ascophyllum* is also an important raw material to produce plant biostimulants. However, we need more knowledge about the microbiome of *Ascophyllum* to understand the role of symbionts in bioactive molecule production. Within the SEABIOZ project, we are studying the roles of endophytes associated with *Ascophyllum* through multi-omics approaches.

Using long-read (Nanopore) and short-read (Illumina) metabarcoding approaches, we examined the composition of the microbiome (bacteria, archaea, fungi, and other eukaryotes) over a year, including thallus age, sex, algal, tissue, and site (three sites at 200-400m distance) as covariables. Our first analyses on a subset of samples indicate a rich bacterial community dominated by Gammaproteobacteria, Alphaproteobacteria and Bacteroidia, which varied across sites and seasons. Fungi were less diverse and dominated by Ascomycota and many yet undescribed amplicon sequence variants (ASVs) belonging to the Dothideomycetes class. Fungal communities were also more stable across time and space. These analyses will be soon completed with the datasets for archaea and other eukaryotes.

The whole microbiome composition analysis will then be complemented with genomic and metagenomic analyses for the same samples, and together these data will be correlated with biological activities of algal extracts and metabolite composition to identify essential microbes, genes, and potentially host-microbe interactions determining the chemical properties of the *Ascophyllum* holobiont.

### The interplay of *Ulva* and its bacteria: acclimation to salinity

van der Loos, L.M.<sup>1,2</sup>, Steinhagen, S.<sup>3</sup>, Willems, A.<sup>2</sup> & De Clerck, O.<sup>1</sup>

<sup>1</sup>Phycology Research Group, Department of Biology, Ghent University, Ghent, Belgium
 <sup>2</sup>Laboratory of Microbiology, Department Biochemistry and Microbiology, Ghent University, Ghent, Belgium
 <sup>3</sup>Department of Marine Sciences-Tjärnö, University of Gothenburg, Sweden
 luna.vanderloos@ugent.be

The green seaweed Ulva is a model system to study seaweed-bacteria interactions. Although it becomes increasingly clear that microbes are of vital importance to their algal host, the impact of environmental drivers on the dynamics of these interactions is little understood. Salinity has been marked as the most important driver of bacterial community composition. In addition, Ulva species are known to have a broad tolerance to salinity. To study how the Ulva host and its bacterial community respond to changes in salinity, our goals were to 1) investigate how salinity structures the composition and function of Ulvaassociated bacterial communities across a natural, environmental salinity gradient, and 2) assess if specific low and high salinity-associated bacteria can aid *Ulva*'s acclimation to changing salinities. We analyzed the taxonomic (n=481 samples) and functional (n=92) patterns of Ulvaassociated bacterial communities across the 2000 km Atlantic-Baltic Sea salinity gradient using both 16S rRNA gene based Oxford Nanopore sequencing and Illumina shotgun metagenomic sequencing. Our results demonstrated that Ulva-associated bacterial taxonomic composition and functional gene composition were strongly structured by both salinity and host species. This led us to the hypothesis that characteristic bacterial communities associated with distinct salinity regions may facilitate the host's adaptation across the environmental gradient. To investigate this hypothesis, we isolated 125 bacteria from Ulva growing in low and high salinity environments in Sweden and Denmark. We then performed laboratory experiments in which axenic (bacteria-free) Ulva fenestrata zoospores were inoculated with specific bacterial communities and exposed to different salinity treatments.

#### Combined *in vivo* and *in situ* genome-resolved metagenomics reveals novel symbiotic nitrogen fixing interactions between noncyanobacterial diazotrophs and microalgae

Chandola, U.<sup>1</sup>, Trottier, C.<sup>1</sup>, Gaudin, M.<sup>2,3</sup>, Manirakiza, E.<sup>1</sup>, Menicot, S.<sup>1</sup>, Louvet, I.<sup>4</sup>, Lacour, T.<sup>5</sup>, Chaumier, T.<sup>1</sup>, Tanaka, A.<sup>6</sup>, Chaffron, S.<sup>2,3</sup> & Tirichine, L.<sup>1</sup>

<sup>1</sup>Nantes Université, CNRS, US2B, UMR 6286, F-44000 Nantes, France.

#### MONDAY 21<sup>st</sup> August 2023 Room « Tali moan »

<sup>2</sup>Nantes Université, École Centrale Nantes, CNRS, LS2N, UMR 6004, Nantes, France.
<sup>3</sup>Research Federation for the Study of Global Ocean Systems Ecology and Evolution, FR2022/Tara Oceans GOSEE, F-75016 Paris, France.
<sup>4</sup>Nantes Université, CNRS, CEISAM, UMR 6230, F-44000 Nantes, France.
<sup>5</sup>Ifremer, PHYTOX, PHYSALG, Rue de l'Ile d'Yeu, BP21105, Nantes Cedex 03, 44311, France
<sup>6</sup>Department of Chemistry, Biology and Marine Science, Faculty of Science, University of the Ryukyus, Okinawa 903-0213, Japan tirichine-l@univ-nantes.fr

The dominance of non-cyanobacteria diazotrophs (NCDs) in marine waters has been demonstrated contradicting the prevailing belief of cyanobacteria dominance. This finding prompts further investigation into the ecological success of these putative heterotrophic bacteria in sunlit oceans. Here, we report an unprecedented finding in the widely used model diatom *Phaeodactylum tricornutum (Pt)* of NCDs sustaining diatom cell growth in the absence of bioavailable nitrogen. We identified *Pt*NCDs using metagenomics sequencing and detected nitrogenase gene *in silico* and/or by PCR. Using metatranscriptomics, we showed the expression of *nifH* genes only under nitrate depletion conditions. We evidenced the presence of nitrogen fixation in *Pt*NCDs and their close genetic affiliation with NCDs from the environment. The prevalence of this type of symbiosis was established through the isolation of NCDs from other microalgae, as well as their detection in the environment and their predicted association with photosynthetic microalgae. Overall, this study provides evidence for a previously overlooked symbiosis using a multidisciplinary model-based approach, which will ultimately contribute to a deeper understanding of the driving forces behind global marine nitrogen fixation.

# The impact of biotic interactions on subcellular structure – an automated 3D imaging approach for marine diatoms

Walde, M.<sup>1</sup>, Camplong, C.<sup>1</sup>, de Vargas, C.<sup>1,2</sup>, Baudoux, A.-C.<sup>1</sup> & Simon, N.<sup>1</sup>

<sup>1</sup>Sorbonne Université, CNRS, Laboratoire Adaptation et Diversité en Milieu Marin UMR7144, Station Biologique de Roscoff, France <sup>2</sup>Research Federation for the Study of Global Ocean Systems Ecology and Evolution, FR2022/Tara GOSEE, Paris, France

\*marie.walde@sb-roscoff.fr

The marine diatoms *Guinardia delicatula* are globally distributed and ecologically successful organisms. They form large annual blooms, which are regulated in part by biotic interactions, including viral infections. Deciphering the impact of such microbial interactions often requires observations on the subcellular scale. However, imaging at high resolution usually comes at the price of low throughput of observable cell numbers. Here, we introduce an innovative approach that combines automated 3D confocal microscopy with quantitative image analysis and physiological measurements to gain insights on both, subcellular and population level.

We initially developed this approach to follow the infection of *Guinardia delicatula* with an RNA virus until the complete lysis of the host and are now using it for the first time in the field. By automatically imaging, classifying, and quantifying hundreds of diatoms across seasons, we aim to quantify their interactions with RNA and DNA viruses, prokaryotes, and other eukaryotes, and measure how the symbiotic landscape is changing over time and in response to biotic and abiotic environmental parameters.

This approach has yielded new detail of the dramatic morphological impact of marine viral infections on their hosts and on how diatoms escape such viral infection. Furthermore, quantitative morphometry of the deformation of essential cellular structures allows for a direct measurement of degraded biomass. In the future, this work will help us to better understand the extent to which viruses and other parasite infect natural diatom populations and their cascading effect on the marine food-web and global biogeochemical cycling.

# Assembly of diatom-associated bacterial communities under phosphate stress

Kayacan, Y.<sup>1</sup>, Mindiola, K.<sup>1</sup>, Chaerle, P.<sup>1</sup>, D'hondt, S.<sup>1,2</sup>, Underwood, G.J.C.<sup>3</sup>, Vyverman, W.<sup>1</sup> & Sabbe, K.<sup>1</sup>

<sup>1</sup>Laboratory of Protistology and Aquatic Ecology, Department of Biology, Ghent University, Ghent, Belgium <sup>2</sup>Phycology Research Group, Department of Biology, Ghent University, Ghent, Belgium <sup>3</sup>School of Life Sciences, University of Essex, Colchester, United Kingdom yeseren.kayacan@ugent.be

Intertidal estuarine sediments are home to productive microphytobenthic communities that form dense biofilms. These biofilms are primarily colonized by motile, epipelic benthic diatoms that secrete extracellular polymeric substances (EPS) which form a matrix that provides organic carbon to heterotrophic bacteria. The production and secretion of EPS are influenced by environmental factors, including nutrient limitation. Compositional differences in EPS produced by diatoms under different nutrient conditions, specifically phosphate limitation, have been shown to drive changes in bacterial community composition via taxonspecific utilization of polysaccharides.

To investigate the effect of P-limitation on the assembly of diatom-associated bacterial communities, a semi-continuous co-culture was monitored for two weeks. The benthic pennate diatom *Cylindrotheca closterium* was cultivated with a natural bacterial community from a marine mudflat (Scheldt Estuary, The Netherlands) under P-rich and P-limited conditions, using both inorganic and organic sources of P. In response to P-stress, diatom growth rate and cell density decreased. Bacterial communities, determined via long-read 16S metabarcoding, were strongly influenced by the limited availability of P. After two weeks of co-culture, P-limited conditions were marked by an increased relative abundance of Rhodobacterales, a group known to specialize in processing low molecular weight diatomderived metabolites. In contrast, P-rich conditions led to an increased relative abundance of Sphingomonadales, which are commonly associated with diatoms. P-stress also led to a higher concentration of monosaccharides in media, suggesting that the composition of diatom EPS, representing a complex pool of organic matter, can create diverse niches for associated bacteria and contribute to the observed changes in bacterial community structure.

# Temporal dynamic of *Ulva* sp. epiphytic bacteria and associated surface metabolome

Paulino, S.<sup>1,2</sup>, Noël, C.<sup>3</sup>, Taupin, L.<sup>1</sup>, Rieusset, L.<sup>1</sup>, Le Blay, G.<sup>2</sup> & Bourgougnon, N.<sup>1</sup>

<sup>1</sup>UBS, Laboratoire de Biotechnologie et Chimie Marines, EMR CNRS 6076, Vannes, France <sup>2</sup>Univ Brest, CNRS, IRD, Ifremer, LEMAR, F-29280 Plouzane, France <sup>3</sup>IFREMER - IRSI - Service de Bioinformatique (SeBiMER), Plouzané, France sauvann.paulino-le-deun@univ-ubs.fr Marine ecosystems are facing increasing pressures from climate change and eutrophication. These changes are causing stresses to marine organisms such as macroalgae, and impact the mutualistic interactions formed with their surface microorganisms, particularly bacteria which occupy the majority of ecological niches on the macroalga surface. The destabilization of this homeostatic relationship in the macroalgal holobiont is likely to lead to an increase of biofouling and to pathologies from the colonization by opportunistic bacteria.

*Ulva* sp. is a green macroalga mainly known for its proliferative abilities during green tide episodes, but it is also a macroalga of economic importance used in various sectors (aquaculture, cosmetic). The aim of this study was to explore the temporal dynamic of the epibacterial communities from *Ulva* sp. and their surface metabolom over a year and a half in south Brittany. The LC-MS based surface metabolomics and 16S rDNA metabarcoding data sets were integrated in a multivariate analysis in order to highlight the positive and negative correlations between microbiota composition, surface metabolites and other potential drivers (biochemical and environmental). Bacterial communities from the algal surface appeared clearly distinct from those in seawater or bedrock and experienced temporal shifts. The main drivers identified were mostly seasonal variations of precipitations, nitrates, salinity and the physiological condition of the algal. A stable core community of bacterial species was also highlighted, with a constant composition at the genus level through time and space.

The integrated multi-omics approach proves to be relevant to study the eukaryotic host – microbiota dynamic system.

## Dynamics of the *Ulva ohnoi* (Chlorophyta, Ulvales) microbiome under different cultivation conditions

Malta, E.-j.<sup>1</sup>, Klonowski, A.M.<sup>2</sup>, Groben, R.<sup>2</sup>, Marteinsson, V.T.<sup>2</sup>, Engelmann, J.<sup>3</sup> & Bolhuis, H.<sup>3</sup>

<sup>1</sup>CTAQUA- Fundación Centro Tecnológico Acuicultura de Andalucía, Spain <sup>2</sup>Microbiology Group, Matís ohf., Reykjavik, Iceland, Country <sup>3</sup>Department of Marine Microbiology and Biogeochemistry, Royal Netherlands Institute for Sea Research, and Utrecht University, Den Hoorn, the Netherlands e.malta@ctaqua.es

It is known that the seaweed microbiome is essential for their growth, however, many questions are still open on how the microbiome varies with cultivation conditions and what effect this might have on seaweed growth and composition. Here, a comparison is made of the microbiome composition of *Ulva* grown in different cultivation systems and its variation in time.

*Ulva ohnoi* was cultivated in earthen ponds (EP), in outdoor tanks (OT) and in 80 L indoor photobioreactors (PBR). Growth rate was monitored weekly and monthly samples were taken for analysis of the algae and water bacterial community (ribosomal RNA amplicon sequencing). In addition, proximate composition of the algae biomass was analyzed.

Growth in EP and OT was mainly driven by irradiance and water temperature, whereas growth in PBRs was generally constant interrupted by collapses. Microbial community differed considerably between the water and the algae for all cultivation systems, with a higher diversity on *Ulva*, but a lower species richness. Important differences were observed also per cultivation condition. Dominant phyla of bacteria in both the water and the *Ulva* were Proteobacteria, Bacteroidetes and Actinobacteria although with important differences in detailed composition. Cyanobacteria were mostly found in the water samples. Clear seasonal shifts were observed at the genus level, including under constant growth conditions, suggesting that the *Ulva* microbiome is not a fixed community but is influence by various factors.

### Integration of Stolen Chloroplasts in Marine Plankton

Rao, A.K.<sup>1</sup>, Yee, D.<sup>1,2</sup>, Juery, C.<sup>1</sup>, LeKieffre, C.<sup>1</sup>, Grundy, A.C.<sup>1</sup>, Chevalier, F.<sup>1</sup>, Jouneau, P.H.<sup>3</sup>, Gast R.<sup>4</sup> & Decelle, J.<sup>1</sup>

<sup>1</sup>Cell and Plant Physiology Laboratory, IRIG, CNRS, CEA, INRAE, Université Grenoble Alpes, 38000 Grenoble, France

<sup>2</sup>*Electron Microscopy Core Facility, European Molecular Biology Laboratory, 69117 Heidelberg, Germany* <sup>3</sup>*Université Grenoble Alpes, CEA, IRIG-MEM, 38000 Grenoble, France* 

<sup>4</sup>Woods Hole Oceanographic Institution, 02543 Woods Hole, Massachusetts, United States raoananya98@gmail.com

Marine microrganisms establish various cellular interactions to acquire carbon energy. These interactions are critical to the functioning of marine ecosystems and their evolution in rapidly changing conditions. Among them, kleptoplastidy (i.e. chloroplast robbery from a photosynthetic organism) is a complex and intriguing phenomenon. A planktonic dinoflagellate from the Ross Sea is known to steal and sequester chloroplasts from the microalga *Phaeocystis antarctica* for several months. This raises many questions on how chloroplasts, which are tightly regulated by the nucleus in their native cell, remain functional and beneficial within a foreign host. This study aims to assess the morphology, physiology and possible metabolic services of the stolen chloroplasts (kleptoplasts).

Electron microscopy imaging revealed that the host retains in a vacuole not only several plastids but also an interconnected network of mitochondria of microalgal origin. The 3D volume reconstructions of several kleptoplasts and chloroplasts from free-living *P. antarctica* show drastic morphological changes. NanoSIMS experiment demonstrated that carbon fixation takes place in kleptoplasts, and PAM measurements were used to track the efficiency of kleptoplasts over time. These different approaches indicate possible maintenance and higher efficiency of kleptoplasts driven by the host dinoflagellate.

Further transcriptome analyses are underway to understand kleptoplast maintenance and mechanism of carbon transfer to the host. The results obtained from this multi-scale study will help decipher the integration of the kleptoplast and subsequently add new insights into the evolutionary process of plastid acquisition in eukaryotes.

# Could algae - bacteria partnership accelerate growth and lipid production of oleaginous benthic diatoms?

Dehel Gamage, N.<sup>1</sup>, Arnaldo, M.D.G.<sup>1</sup>, Mossion, A.<sup>1</sup>, Wielgosz-Collin, G.<sup>1</sup>, Lebeau, T<sup>2</sup>, Méléder, V.<sup>1</sup>

<sup>1</sup>Institute of Substances and Organisms of the Sea (ISOMer), Nantes Université, France <sup>2</sup>Laboratory of Planetology and Geosciences, Nantes Université, France nadeeshani.dehel-gamage@etu.univ-nantes.fr

Microalgae-bacteria interaction has recently gained particular attention as some research studies have demonstrated the positive impact of these associations on the growth and interest metabolites production of microalgae such as lipids and pigments. However, a deeper understanding about the bacterial structural diversity associated with different microalgae, the nature of their relationships, chemical mediated exchanges between organisms and how the exchange of chemical compounds impact on the growth and interest metabolites production of microalgae is still lacking.

#### MONDAY 21<sup>st</sup> August 2023 Room « Tali moan »

With the hypothesis, the interaction between marine benthic diatoms and naturally occurring bacteria under the laboratory culture conditions could increase the growth and lipid production of marine benthic diatoms, our project aims to optimize the lipid production of two oleaginous benthic diatoms: *Amphora* sp., *Staurosira* sp. by diatom-bacteria association. The structural dynamics of bacteria surrounding these two benthic diatoms, nature of their relationship (e.g. mutualism, parasitism) and the exometabolites produced by bacteria which could play a pivotal role on the growth and intracellular lipid production of benthic diatoms as well as co-culturing of benthic diatoms with selected bacteria strains will be carried out using the biofilm photobioreactor (e.g. a porous substrate photobioreactor, PSBR) which is specifically designed for culturing benthic diatoms. Identification of isolated bacteria by sequencing based 16S rDNA, measuring the biomass and photosynthetic efficiency of benthic diatoms using PAM- fluorometry, analyzing the lipid profile of benthic diatoms with GC-MS and the analysis of exometabolites in the culture media using UHPLC/Q-TOF-MS will be conducted as prioritized activities.

Hopefully, integrating the essence of ecologically important associations between autotrophic benthic diatoms and heterotrophic bacteria in cropping systems could positively result in the growth aspects of biotechnology related to the pharmaceutical, nutraceutical and biofuel industries in the future.

### How does light affect the presence and antibacterial activity of *Phaeobacter* in the *Ulva ohnoi* holobiont?

Qui-Minet, Z.N.<sup>1</sup>, Wichard, T.<sup>2</sup>, Del Olmo, G.<sup>1</sup>, Ruiz, P.<sup>1</sup>, Cremades, J.<sup>3</sup> & Pintado, J.<sup>1</sup>

<sup>1</sup>Marine Ecology and Resources Group. Institute of Marine Research (IIM-CSIC), Galicia, Spain. <sup>2</sup>Jena School for Microbial Communication, Institute for Inorganic and Analytical Chemistry, Friedrich Schiller University Jena, Jena, Germany

<sup>3</sup>Coastal Biology Research Group (BioCost). CICA - Interdisciplinary Center for Chemistry and Biology, University of A Coruña, Galicia, Spain. zqui@iim.csic.es

*Ulva* provides an important niche for biofilm-forming bacteria, such as the *Phaeobacter* genus, producing antibiotic compounds like TDA (tropodithietic acid) with antagonistic activity towards fish pathogens, including *Vibrio anguillarum*. It is, thus, interesting from an economic point of view to understand the environmental conditions that promote the presence of *Phaeobacter* on the surface of *Ulva* used in Integrated Multi-Trophic Aquaculture (IMTA), including the application as a biofilter of fish effluents.

Upon inoculation of *U. ohnoi* with *Phaeobacter* sp. 4UAC3, it is maintained when *U. ohnoi* is kept in the dark, however, *Phaeobacter* sp. eventually disappears when the algae are growing under light in a regular photoperiod. Our study focused on the effect of light on (i) the metabolome of the *Ulva* holobiont in the two light regimes, (ii) the production of TDA by

*Phaeobacter* forming a biofilm in *U. ohnoi*, (iii) the inhibition of *V. anguillarum* by *Phaeobacter* biofilms in *U. ohnoi*. Our results show (i) significant differences in the metabolome produced by the *Ulva* holobiont in the two light regimes, which may be responsible for the disappearance of *Phaeobacter* in the *Ulva* biofilm, (ii) an up-regulation of TDA on *U. ohnoi* cultivated in the dark, (iii) inhibition of *V. anguillarum* by *Phaeobacter* when forming a biofilm on *U. ohnoi*. Our results highlight the challenge of mastering seaweed physiology from the holobiont perspective for applications in aquaculture.

# Natural biocontrol of a *Porphyra sp.* epiphyte on farmed *Gracilaria chilensis* by a Pythiosis outbreak

Murúa, P.<sup>1,2</sup>, Patiño, D.<sup>1</sup> & Muñoz L.<sup>1</sup>

<sup>1</sup>Laboratorio de Cultivo de Macroalgas y Ficopatología FICOPAT, Instituto de Acuicultura, Universidad Austral de Chile, Sede Puerto Montt, Chile <sup>2</sup>The Scottish Association for Marine Science, Scottish Marine Institute, Culture Collection for Algae and Protozoa, Oban, PA37 1QA, United Kingdom liliana.munoz@uach.cl

*Gracilaria chilensis* (a.k.a. pelillo) is the most produced seaweed in Chile and Latin America, yet its cultivation has historically faced lots of pest-associated constrains that threat its profitability and sustainability. Pests show temporal cycles of recruitment, growth and death/senescence, variation usually linked with sharp changes in environmental factors. Here we report the appearance of *Porphyra* epiphytic on farmed *Gracilaria*, recruits that cover a significant proportion of its basophyte in few weeks in a suspended set-up in spring. *Porphyra* development proliferates until a filamentous fungal-like organism colonizes its tissues, infecting a wide proportion of them. After this outbreak *Porphyra* collapses, disappearing in few weeks from farmed *Gracilaria*. Observations of diseased individuals, and subsequent isolation and marker-assisted taxonomy of the pathogen, provide evidence for ID this organism as *Pythium porphyrae*, the aetiological agent for the red rot disease in commercial nori/gim in Asia. This is the first reported case in Chile for natural biocontrol of pest in *Gracilaria* aquaculture, suggesting and unknown -yet considerable- cryptic biodiversity acting as natural regulators of Gracilaria pests.

# Isotopic tracing reveals single-cell assimilation of a macroalgal polysaccharide by marine Flavobacteria and Gammaproteobacteria

Thomas, F.<sup>1</sup>, Le Duff, N.<sup>1</sup>, Wu, T-D.<sup>2,3</sup>, Cébron, A.<sup>4</sup>, Uroz, S.<sup>5</sup>, Riera, P.<sup>6</sup>, Leroux, C.<sup>7</sup>, Tanguy, G.<sup>8</sup>, Legeay, E.<sup>8</sup>, Guerquin-Kern, J-L.<sup>2,3</sup>

<sup>1</sup>Sorbonne Université, CNRS, Integrative Biology of Marine Models (LBI2M), Station Biologique de Roscoff (SBR), 29680 Roscoff, France

<sup>2</sup>Institut Curie, Université Paris-Saclay, Paris, France

<sup>3</sup>Université Paris-Saclay, INSERM US43, CNRS UMS2016, Multimodal Imaging Center, Orsay, France

<sup>4</sup>Université de Lorraine, CNRS, LIEC, F-54500 Nancy, France

<sup>5</sup>Université de Lorraine, INRAE, UMR1136 « Interactions Arbres-Microorganismes », F-54280 Champenoux, France

<sup>6</sup>Sorbonne Université, CNRS, UMR7144, Station Biologique de Roscoff (SBR), 29680 Roscoff, France

<sup>7</sup>CNRS, Sorbonne Université, FR2424, Metabomer, Station Biologique de Roscoff, 29680 Roscoff, France

<sup>8</sup>CNRS, Sorbonne Université, FR2424, Genomer, Station Biologique de Roscoff, 29680 Roscoff, France fthomas@sb-roscoff.fr

Algal polysaccharides constitute a diverse and abundant reservoir of organic matter for marine heterotrophic bacteria, central to the oceanic carbon cycle. We investigated the uptake of alginate, a major brown macroalgal polysaccharide, by microbial communities from kelp-dominated coastal habitats. Alginate amendments induced a decrease in bacterial diversity and a marked compositional shift towards copiotrophic bacteria. We traced <sup>13</sup>C derived from alginate into specific bacterial incorporators and quantified the uptake activity at the single-cell level, using halogen *in situ* hybridization coupled to nanoscale secondary ion mass spectrometry (HISH-SIMS) and DNA stable

isotope probing (DNA-SIP). Cell-specific alginate uptake was observed for *Gammaproteobacteria* and *Flavobacteriales*, with carbon assimilation rates ranging from 0.14 to 27.50 fg C  $\mu$ m<sup>-3</sup> h<sup>-1</sup>. DNA-SIP revealed that only a few initially rare *Flavobacteriaceae* and *Alteromonadales* taxa incorporated <sup>13</sup>C from alginate into their biomass, accounting for most of the carbon assimilation based on bulk isotopic measurements. These results highlight the high degree of niche specialization in heterotrophic communities and help constraining the quantitative role of polysaccharide-degrading bacteria in coastal ecosystems.

# *Phaeocystis/Acantherea* symbiosis: genomic and transcriptomic insight of core transporter genes.

Juery, C.<sup>1</sup>, Margaret, B., Yee, D., Chevalier, F., Allen, A., Füssy, Z. & Decelle, J.<sup>2</sup>

<sup>1,2</sup>Cell & Plant Physiology Laboratory, UMR 5168 CEA-CNRS-Univ. Grenoble Alpes – UMR1417 INRAE, Grenoble, France

\*caroline.juery@cea.fr

Planktonic symbiosis in the ocean, involving a large diversity of protists has been the poor stepchild of studies on symbiotic interactions despite its crucial importance in biogeochemical cycling for marine ecosystems understanding (Decelle *et al.* 2015). In the past decade, Johan Decelle and Margaret Brisbin research work started to decipher the intimate relationship between the microalgae *Phaeocystis* and the Acantherae (Decelle *et al.* 2012, Brisbin *et al.* 2018, Decelle *et al.* 2019, Uwizeye *et al.* 2021). In the last article cited, they establish the first set of differentially expressed genes of *Phaeocystis* within the symbiosis. However, contrary to the emblematic coral-reef symbiosis for which some article present the putative currency and transporters involved in the exchanges (Burriesci *et al.* 2012, Maor-Landow *et al.* 2019), no research has been done yet on that question for Phaeocytis/Ancantharea symbiosis.

In this study, we created a complete dataset of field and experimental samples for the study of Single-Cell gene expression in order to identify transporter genes that might be involved in metabolic exchanges. After having inventoried the transporters gene set of *Phaeocystis* at the genomic level, we identified  $\sim$ 170 up and  $\sim$ 300 down regulated transporter genes in the symbiosis. As for the cnidarian/symbiodinium symbiosis (Maor-Landow *et al.* 2023), we highlight the SWEET transporter gene family as involved in this interaction.

The characterization of the putative substrates of these transporters in our lab is now one of the challenges we aim at take up to reveal the metabolic fluxes occurring through this fascinating Photosymbiosis.

# Characterising the bacterial pathobiome of diatoms inhabiting a productive temperate coastal ecosystem

Branscombe, L.<sup>1,2</sup>, Wilson, W.<sup>1,2</sup>, Cunliffe, M.<sup>1,2</sup> & Helliwell, K.E.<sup>1,3</sup>

<sup>1</sup>Marine Biological Association, Citadel Hill Plymouth, PL1 2PB, <sup>2</sup>University of Plymouth, Plymouth, UK <sup>3</sup>University of Exeter, Exeter EX4, 4QD Katherine.helliwell@mba.ac.uk; k.helliwell@exeter.ac.uk

Diatoms are globally abundant eukaryotic algae that frequently form extensive blooms in aquatic ecosystems. Certain bacteria behave antagonistically towards diatoms, killing or inhibiting their growth.

#### MONDAY 21<sup>st</sup> August 2023 Room « Tali moan »

Despite their crucial implications to diatom health and bloom control, fundamental insight of the population structure and dynamics of antagonistic bacteria in natural ecosystems is lacking. We report an ecosystem level assessment of the diversity of diatom antagonistic bacteria, employing a plaqueassay approach sampling monthly in the Western English Channel (WEC) over an annual cycle. We identified 18 plaque-forming bacteria spanning four classes and ten bacterial orders, across five diatom hosts. Several bacteria, most notably the Roseobacter Ponticoccus alexandrii, were isolated recurrently, indicative of a persistent environmental presence. For many of these isolates, antagonistic activity against diatoms has not previously been reported. We verified growth inhibitory effects against diatoms for eight such bacteria. In most instances antagonism was facultative, detected only when bacteria were pre-grown on diatom-derived biomass. Notably, cooccurrence analyses revealed that peaks in antagonist activity of several bacteria corresponded with a winter diatom bloom in the WEC. Moreover, examination of Tara Oceans data revealed global-scale distribution patterns of WEC antagonists, ranging from the Mediterranean Sea to the Arctic Ocean. Detection of co-occurrences of bacteria with diatom hosts in several ocean regions were also observed, for interactions verified in the laboratory. Thus, by employing an ecosystem approach our study has revealed that multiple bacterial antagonists with overlapping host specificities reside in this productive coastal ecosystem, exhibiting peaks in abundance that correspond with diatom bloom events.

# Metabolic co- evolution: the case of the oxylipin pathway in a host - endophyte interaction between brown macroalgae

Zonnequin, M.<sup>1</sup>, Delage, L.<sup>1</sup>, Hamon-Giraud, P.<sup>2</sup>, Leroux, C.<sup>3</sup>, Veillet, F.<sup>1,4</sup>, Badis, Y.<sup>1</sup>, Cock, J.M.<sup>1</sup>, Siegel, A.<sup>2</sup>, Leblanc, C.<sup>1</sup> & Markov, G.<sup>1</sup>

<sup>1</sup>Sorbonne Université, CNRS, Integrative Biology of Marine Models (LBI2M), Station Biologique de Roscoff (SBR), 29680 Roscoff, France

<sup>2</sup>Univ Rennes, Inria, CNRS, IRISA, F-35000 Rennes, France

<sup>3</sup>Sorbonne Université, CNRS, FR2424, Metabomer-Corsaire, Station Biologique de Roscoff (SBR), 29680 Roscoff, France

<sup>4</sup>*AMU, CEA, CNRS, BIAM Cadarache, 13108 Saint-Paul-lez-Durance, France* maelle.zonnequin@sb-roscoff.fr

The evolution of organisms results in continuous changes in metabolic pathways. This phenomenon is described as metabolic drift. We hypothesize that biotic interactions may be a driving force by fostering metabolic plasticity. Brown algae offer unique model systems to address this question, with hostendophyte species pairs belonging to the same phylum. These pairs enable the reconstruction of the establishment of interactions between the metabolic pathways of both partners dating back to their common ancestor. The phylogenetic proximity between Laminariales and their endophytic Ectocarpales increases the probability that orthologous enzymes have identical catalytic activities, allowing compensation phenomena and loos of genes by endophytes. Comparison of genome-scale metabolic networks has identified metabolic reactions that may have been lost in these endophytes. Some of these reactions are part of the oxylipin pathway which is induced during defense responses in the context of biotic interactions. One of the losses concerns a gene encoding a CYP5164 ensyme, a member of a multigenic subfamily that has diversified in Laminariales and Ectocarpales. Metabolic profiling of a CRISPR konck-out mutant for the CYP5164B1 gene in a free-living Ectocarpale indicates that there are differences in the global metabolomic profile compared to the wild type strain. Further targeted profiling and production of recombinant CYP5164s protein are ongoing enabling their biochemical activities to be characterized and validates in vitro and is expected to identify species-specific substrates.

Together these approaches will contribute to deciphering the biosynthesis and evolutionary origin of oxylipin signals during long-term biotic interactions between closely related brown algae.

#### Deciphering the domestication of a seaweed holobiont

Faugeron, S.<sup>1,2</sup>, Guillemin, M-L.<sup>1,3</sup>, Trefault, N.<sup>4</sup> & Camus, C.<sup>5</sup>

<sup>1</sup>IRL3614 Evolutionary Biology and Ecology of Algae, CNRS, Sorbonne Université, Pontificia Universidad Católica de Chile, Universidad Austral de Chile, Roscoff, France
<sup>2</sup>Núcleo Milenio MASH, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile
<sup>3</sup>Núcleo Milenio MASH, Universidad Austral de Chile, Valdivia, Chile
<sup>4</sup>Núcleo Milenio MASH, Centro GEMA, Universidad Mayor, Santiago, Chile
<sup>5</sup>Núcleo Milenio MASH, Centro I-Mar, Universidad de los Lagos, Puerto Montt, Chile
<sup>s</sup>faugeron@bio.puc.cl

Deciphering the drivers of seaweed domestication requires understanding the influence of the microbial biofilm, a specific component of the biotic environment that interacts with the seaweed host in a coordinated manner, making the holobiont the relevant ecological and evolutionary unit. Here, we investigated the changes of the microbial community associated with the red seaweed *Gracilaria chilensis*, from natural populations to farms, and the effects they have on seaweed traits. We tested the hypothesis that both the environment (natural versus farm) and the seaweed's genetic background (wild versus domesticate) could determine the composition of the microbiota, by comparing the 16S diversity and the genetic diversity of the host seaweed (based on 87 SNPs) between three farms and three natural populations. We also tested the hypothesis that the microbial composition interacts with the seaweed's genetic background to determine the expression of seaweed traits. To do so, we transplanted microbial communities between 10 farmed and 10 wild seaweed strains and measured their physiological performance (i.e. photosynthetic efficiency) and response to epiphytes (i.e. settlement and germination rate of the red filamentous algae *Acrochaetium* sp on *G. chilensis*' surface).

#### A Jekyll-and-Hyde seaweed pathogen

Weinberger, F.<sup>1</sup>, Chapuis, L.<sup>1,2</sup>, Borchert, E.<sup>1</sup>, Engelen, A.H.<sup>3</sup>, Saha, M.<sup>4,1</sup> & Wiese, J.<sup>1</sup>

<sup>1</sup>GEOMAR Helmholtz-Centre for Ocean Research, Kiel, Germany <sup>2</sup>IMBR Sea, Ghent University, Belgium <sup>3</sup>CCMAR, Universidade do Algarve, Faro, Portugal <sup>4</sup>Plymouth Marine Laboratory, U.K. fweinberger@geomar.de

Thallus bleaching is frequently observed in the red seaweed *Gracilaria vermiculophylla*, with *Pseudoalteromonas arctica* and some other bacterial taxa a known inducers of the symptom. These pathogens are frequently associated with virtually healthy host specimens and their virulence was demonstrated to be controlled by an array of other bacterial taxa. Several of these protectors are also members of the genus *Pseudoalteromonas*, including *P. ulvae*, *P. atlantica* and *Pseudoalteromonas* sp. strain GNORD11, a species that has not yet been described.

We here focused on the two related species *P. arctica* and *Pseudoalteromonas* GNORD11 and aimed to investigate their respective effects as pathogen and protector. After the natural protective microbiome

component had been disrupted by treatment with antibiotics the medium of healthy hosts was inoculated with only one or both strains at differently adjusted cell densities. *P. arctica* induced disease symptoms when more than 20 cells ml<sup>-1</sup> were inoculated. Surprisingly, the supposed protector GNORD11 also induced disease symptoms when between 30 and 30 000 cells ml<sup>-1</sup> were inoculated. However, at higher cell densities no virulence was observed and GNORD11 even prevented the virulence of *P. arctica*. GNORD11 exhibited an antibiotic effect on *P. arctica* and genome sequencing hinted at its capacity to produce various potential virulence regulators, such as 2-heptyl- 4quinolone, several other quorum sensing mediators and a wide spectrum of confirmed and potential antimicrobial compounds. Strain GNORD11 thus behaves according to the Jeckylland-Hyde principle, which predicts that certain symbionts may shift between mutualistic and antagonistic behaviour toward their host.

# Kelp microbiomes benefits to the test: bacteria isolation, characterization, and tested for influence on kelp resilience to global change stress conditions.

Cojoc, L.C., Indart, A., Barreto, L., Aires, T., Martins, N., Serrão, E.A. & Engelen, A.H.

CCMAR, Portugal aengelen@ualg.pt

In temperate marine regions, kelps are dominant shallow water seaweed habitat formers, founding one of the most productive ecosystems in the world. The impacts of anthropogenic climate change pose a major threat to kelp forests, affecting their physiology and survival, and driving declines in abundance in many regions globally. Interactions with their associate microbial communities are fundamental for kelp health, defense, vitamin provision, and perhaps even climate resilience. Therefore, microbiome engineering can potentially enhance kelp performance and/or confer resistance or resilience to future environmental conditions.

Here, we evaluated if bacteria could contribute to the kelp health and resistance to increased temperatures of juvenile *Laminaria ochroleuca* sporophytes from two contrasting habitats. A wide variety of in total 65 bacteria was isolated, on Marine Agar 2216 and R2A media, from *Laminaria ochroleuca* sporophytes and gametophytes originating from Portugal, France, Italy, and Morocco. Catalase activity was demonstrated for 33 of the isolates. We selected a bacterial consortium composed of ten bacteria: two *Halomonas.*, *Maribacter*, *Sulfitobacter* and *Paraglaciecola*, and a *Zobelia*, and a *Colwellia*. Young sporophytes from Morocco and Italy were exposed to a temperature treatment of 12, 18, 22, 23, and 24°C with and without this bacterial consortium to test for thermal tolerance benefits of the added consortium. The physiological response was assessed by determining host' growth and photosynthetic performance. The findings suggest a population-specific response regarding sporophytes.

### SYMPOSIUM 13 "Algae and ecosystem functioning: the genomics perspective"

### **KEYNOTE**

# Viral infection dynamics in marine diatoms across environmental and ecophysiological gradients.

#### Kranzler, C.

Faculty of Life Sciences, Bar-Ilan University, Israel chana.kranzler@biu.ac.il

Diatoms are a diverse and widespread group of aquatic, photosynthetic, eukaryotic microorganisms. Key players in the global carbon cycle, diatoms contribute  $\sim 20\%$  of the primary production on the planet. With an obligate silicon requirement for cell wall formation, diatoms also facilitate substantial export of particulate organic carbon out of the surface into the deep ocean. While viruses are considered catalysts of ecological, evolutionary, and biogeochemical process in the ocean, very little is known about the unique viruses that infect diatoms. Using a combination of molecular, physiological, and biogeochemical techniques, we are exploring diatom host-virus interactions. In natural phytoplankton communities, we are applying metatranscriptomics to explore both diatom transcriptional frameworks together with cell-associated diatom virus populations. In parallel, we are interrogating viral infection dynamics in the bloom-forming, centric diatom, Chaetoceros tenuissimus, an emerging model system for diatom host-virus interactions, to better understand the cellular frameworks that underscore viral infection in diatoms. Our work has identified diverse diatom host-virus infection dynamics, with dramatic changes in viral diversity, virus production and virus-mediated mortality in response to different types of nutrient limitation. These findings contextualize diatom viruses within the ecophysiological framework of nutrient availability and highlight the dynamic role that nutrient-driven host-virus interactions play in determining the fate of diatom carbon, silica and associated elements in the ocean.

### ORALS

#### Prostaglandins in marine diatoms

Di Dato, V.<sup>1</sup>, Di Costanzo, F.<sup>1</sup>, Orefice, I.<sup>1</sup>, Sepe, R.M.<sup>1</sup>, Magnabosco, C.<sup>1</sup>, Montresor, M.<sup>1</sup>, Aiese Cigliano, R.<sup>2</sup> & Romano, G.<sup>1</sup>

<sup>1</sup>Stazione Zoologica Anton Dohrn, Ecosustainable Marine Biotechnology department, Naples, Italy <sup>2</sup>Sequentia Biotech SL, Barcelona, Spain valeria.didato@szn.it

Diatoms are very diverse unicellular microalgae that play a fundamental role in the functioning of global ecosystems contributing to about 20% of annual global carbon fixation, underpinning major aquatic

#### MONDAY 21<sup>st</sup> August 2023 Room « Tali friz »

food webs and driving global biogeochemical cycles. They produce secondary metabolites that serve as chemical defence and communication, with relevant differences even at the clone level.

In 2017 we demonstrated the presence of prostaglandins (PGs) in two diatom species, *Skeletonema marinoi* and *Thalassiosira rotula*. While in mammals, PGs play multiple signalling roles in inflammation, immune responses, tumour growth, etc, their role in the marine environment is still undisclosed. Their presence in diatoms is intriguing and opens new questions about their role in single cell organisms, also questioning about the evolutionary history of these important eicosanoids.

We have shown their release in the growth media and the differential expression of the enzymes responsible for their synthesis among species and clones of a same species, among different culture conditions and along diatoms growth curve. In the frame of the Italian project named "CRIMAC", by applying different technologies (NGS, ddPCR, chemistry assays etc.) on different type of samples (laboratory culture, natural samples, dormant cultures etc.) thanks to genome sequencing we are characterizing the enzymes involved in the pathway while, at the same time, assessing the ability of the different diatom species to produce prostaglandins in a natural environment and the role of those molecules in relation to formation of dormant status, to predators defence and adaptation to physico-chemicals parameter, i.e. light, temperature and pH..

### Iron monopolization versus community service: the two faces of cyanobacterial beta-hydroxy aspartate lipopeptides

Falcao, B.<sup>1,2</sup>, Galica, T.<sup>1</sup>, Štenclová, L.<sup>2,3</sup>, Martínez Yerena, J.A.<sup>1,2,3</sup>, Mareš, J.<sup>1,2,3</sup>, Kozlíková, E.<sup>3</sup> & Hrouzek, P.<sup>1</sup>

<sup>1</sup>Institute of Microbiology, CAS, Novohradská 237, Třeboň, 379 01 Czechia <sup>2</sup>Faculty of Science, University of South Bohemia, Branišovská 1760, České Budějovice, 05 Czechia <sup>3</sup>Institute of Hydrobiology, Biology Centre CAS, Na Sádkách 702/7, České Budějovice, 05 Czechia stenclova.lenca@gmail.com

In living systems, iron serves as an essential cofactor for the basal metabolism. Scarcity of biologically accessible iron can be a limiting factor for the growth of microorganisms in marine environments and terrestrial microhabitats with steep microgradients such as arid soils. Cyanobacteria are an important component of the soil crust communities in arid regions, especially in terms of primary production and excretion of biologically active compounds. Among these, siderophores are capable to chelate desired ferrous ions and facilitate their uptake by the recipient organisms.

Recently, biosynthetic gene clusters for siderophores bearing two iron-chelating  $\beta$ -hydroxy aspartate residues were discovered in three phylogenetically distant cyanobacterial strains and designated as cyanochelins. The intriguing feature of cyanochelins is their ability to undergo UV-dependent photolysis, releasing the bioavailable Fe<sup>2+</sup> and presumably providing it to the microbial community. The potential recipients possibly occur among both the cyanobacteria and other bacterial taxa.

Our genome mining suggested a wider presence of cyanochelin gene clusters within Leptolyngbyaceae, a cyanobacterial group containing simple-filamentous taxa frequently present in soil crusts. Our survey of soil-crust samples discovered four candidates producing  $\beta$ -hydroxy aspartate lipopeptides, including novel compounds. Taxonomic identity of the cyanochelin producers was verified and strains were isolated for characterization of new cyanochelin variants and co-cultivation experiments. The microbial community composition and biosynthetic capability was assessed via whole metagenome sequencing. In following experiments, the analysis of siderophore transporters in genomes of co-occurring microbial taxa, and isolation of cyanochelin-dependent cohabitants can tell us more about processes of iron cycling in the soil-crust environments.

### **KEYNOTE**

#### Exploring and manipulating the Saccharina latissima microbiome

Burgunter-Delamare, B., Laugier, J., Boyen, C., Dittami, S.

Sorbonne Université, CNRS, Integrative Biology of Marine Models (LBI2M, UMR 8227), Station Biologique de Roscoff, 29680 Roscoff, France

*Saccharina latissima* is a canopy-forming brown alga of economic and ecological in Europe. To better understand its biology and ecology, we need more data on its associated microbiota and the importance of host-microbiota inactions in this species.

This talk will summarize various aspects of our recent research on the microbiome of *S. latissima*. The first part will give an overview of the taxonomic composition of the S. latissima microbiome, its core components, and its variability across different parts of the algae, seasons, and regions. We will also highlight characteristic microbial signatures for healthy algae vs. algae in poor health and explore to what extent the microbiome is vertically transmitted vs. defined by the environment. The second part will focus on co-cultures carried out in laboratory conditions to elucidate the beneficial and harmful effects of inoculation with selected *S. latissima*-derived microbes. While the outcomes of these experiments were highly variable, we observed an overall negative correlation between host growth and the detection of AI-1-type quorum-sensing (QS) compounds in the culture medium. Furthermore, we showed that the medium from algae exposed to stress can elicit the production of QS compounds in some bacteria.

Together, these data contribute to our understanding of the variability of the *S. latissima* microbiome and establish a basis for further experimental evaluations of algal-bacterial interactions and microbiome manipulations in this system.

### ORALS

# Exploring the Role of Microbiome and Epigenetics in Shaping the Response of *Phaeodactylum tricornutum* to Hypersalinity

Wu, Y., Chaumier, T. & Tirichine, L.

Nantes Université, CNRS, US2B, UMR 6286, F-44000 Nantes, France yue.wu1@etu.univ-nantes.fr

*Phaeodactylum tricornutum*, unlike other diatoms, exhibits unique morphological plasticity, capable of existing in different morphotypes such as fusiform, triradiate, cruciform and oval, which can be influenced by environmental conditions. Salinity is a significant determinant in the geographical distribution of marine and estuarine species, and is one of several major environmental factors that affect the complex bacterial communities associated with their hosts. In our study, we investigated the effect of bacterial communities on the acclimation of *P. tricornutum* to hypersaline conditions. Our results demonstrate that *P. tricornutum* depends on its associated microbiome to survive a transfer to hypersaline conditions. Cultures deprived of their bacterial communities do not survive in hypersaline environments. We found that changes in salinity concentrations significantly affect bacterial community composition of two accessions of *P. tricornutum* with a shift towards halophile species. Phenotypic plasticity is a critical aspect of an organism's response to environmental changes and survival, yet the underlying mechanisms remain elusive. Ecological epigenetics has emerged as a promising field that

#### MONDAY 21<sup>st</sup> August 2023 Room « Tali friz »

may offer mechanistic insights into phenotypic plasticity and fitness. Specifically, DNA methylation may provide the molecular basis for the plasticity of traits that are critical for an organism's ecological success. Our results showed that different accessions of *P. tricornutum* exhibited distinct morphotype traits in response to hypersalinity versus normal salinity. Interestingly, both accessions exhibited a tendency towards DNA hypomethylation under hypersalinity. However, whether these epigenetic changes are directly involved in the phenotypic plastic response of diatoms remains to be investigated.

# A global genomics study of *Raphidiopsis raciborskii*, demonstrating the power of culture collection collaborations

Willis, A. and a global network of participants<sup>§</sup>.

Australian National Algae Culture Collection, CSIRO, Australia anusuya.willis@csiro.au

*Raphidiopsis raciborskii* is a global cyanobacterium, including both toxic and non-toxic strains. It occurs, and frequently blooms, in freshwater ecosystems across tropical and temperate environments. Local scale studies have shown high physiological and genomic diversity between strains of *R. raciborskii*, indicating possible rapid adaptation to new environments and resilience to environmental changes.

To investigate the global genomic diversity of *R. raciborskii* we collaborated with culture collections and researchers to collect  $\sim$ 80 strains from 22 countries, spanning the continents America, Europe, Africa, Asia, and Australia.

Comparative genomics show a small core genome and a large variable shell genome, suggesting a flexible genome evolution strategy. However, many gene presence/absences appear to be redundant and overall metabolic functions are similar across all genomes. There is a lack of unique features within each cluster and genome structural changes appear haphazard across the genome. The genomes are also rich in anti-phage systems, with over 500 CRISPR arrays, suggesting high rates of phage and plasmid interaction. Speciation is occurring through geographic isolation, and three distinct species clusters were evident, indicating taxonomic changes and new species descriptions are needed within *Raphidiopsis*. The *R. raciborskii* global pangenome reveals a species with a flexible genome and local adaptation without local functional differences. This study demonstrates the power of culture collection collaborations and the benefits of global analysis.

§global network of participants:

*Anusuya Willis* and *Ian Jameson;* Australian National Algae Culture Collection, CSIRO, Hobart, TAS, Australia - *Catharina Alves-de-Souza;* Algae Resource Centre, University of North Carolina Wilmington, USA - *Cecile Bernard* and *Charlotte Duval;* Muséum National d'Histoire Naturelle, Paris, France - *Masanobu Kawachi* and *Haruyo Yamaguchi;* National Institute for Environmental Studies (NIES), Japan - *Ruth N. Levy-Kaplan;* Israel National Culture Collection of Algae, Kinneret Limnological Laboratory, Israel Oceanographic & Limnological research, Israel - *Alescia Cullen* and *Brett Neilan;* University of Newcastle, Australia - *Maxine A. D. Mowe, Darren C. J. Yeo;* National University of Singapore, Singapore - *Simon M. Mitrovic;* University of Technology Sydney, Australia - *Dariusz Dziga;* Laboratory of Metabolomics, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Gronostajowa 7, 30387 Krakow, Poland - *Mikołaj Kokociński;* Department of Hydrobiology , Faculty of Biology, Adam Mickiewicz University, Uniwersyteu Poznańskiego 6, 61-614 Poznań, Poland - *Jun Yang* and *Fengjiao Tan;* Aquatic EcoHealth Group, Fujian Key Laboratory of Watershed Ecology, Key Laboratory of Urban Environment and Health, Institute of Urban Environment, Chinese Academy of Sciences, Xiamen 361021, China - *Muriel Gugger;* Cyanobacteria Culture Collection, Pasteur Institute, Paris, France - *Jason Woodhouse;* Dept. of Experimental Limnology, Leibniz Institute of Freshwater Ecology and Inland Fisheries (IGB), 16775 Stechlin, Germany

### SYMPOSIUM 11 "Changing distribution patterns and new ecosystems"

### **KEYNOTE**

### On the track of causes for fish-killing algal blooms

Edvardsen, B.

University of Oslo, Department of Biosciences, Norway. bente.edvardsen@ibv.uio.no

Harmful algal blooms (HABs) in marine, brackish and fresh waters resulting in fish-kills occur worldwide and may lead to great economic and ecological losses, as well as suffering for the fish. Farmed fish in pens and ponds are especially prone as they cannot escape from the blooms. Many ichthyotoxic blooms are sporadic events and the causes and triggering factors of these HABs are often poorly understood. Knowledge on the responsible algae and their physiology, ecology and environmental factors promoting their growth are required to understand these events. Bloom formation may also be linked to their toxicity through a relaxation of the top-down control from grazers, pathogens or competitors. We thus also need to understand how toxin production or toxicity are affected by various environmental factors to be able to understand causes of HABs. Knowledge about the responsible toxin(s) and their mode of action may however be limited and difficult to quantify. In this presentation I will compare ichthyotoxic haptophytes and look at similarities and differences in their bloom formation, ecology, physiology and toxicity in order to better understand causes for some of these blooms, especially of the little studied species *Chrysochromulina leadbeateri*.

### ORALS

# *In situ* and *in vitro* transcriptomic response of picophytoplankton *Pelagomonas calceolata* to low nitrate conditions

Guérin, N.<sup>1,2</sup>, Carradec, Q.<sup>1,2</sup> & Wincker, P.<sup>1,2</sup>

<sup>1</sup>Génomique Métabolique, Genoscope, Institut François Jacob, CEA, CNRS, Univ Evry, Université Paris-Saclay, 91057, Evry, France

<sup>2</sup>Research Federation for the Study of Global Ocean Systems Ecology and Evolution, R2022/Tara Oceans GO-SEE, 3 rue Michel-Ange, 75016, Paris, France nguerin@genoscope.cns.fr

nguermagenescope.ens.n

Among the outcomes of climate change, global ocean warming increases stratification and limits nutrient supplies in surface waters. Thus, oligotrophic areas will grow and affect phytoplankton abundance and distribution. Photosynthetic picoeukaryotes (PPE) are vital primary producers in low-nutrient waters and should benefit from this phenomenon. However, we still need model organisms, representatives of pelagic environments, to understand their acclimation to nutrient limitation.

*Pelagomonas calceolata* is a cosmopolite and abundant pelagic PPE. We hypothesize that transcriptomic acclimation to environmental changes can explain its ecological success. The recent

genome of *P. calceolata* and environmental meta-omics data are assets to study its gene expression globally. Besides, *P. calceolata* is cultivable, allowing experiments in controlled conditions to explore physiological and transcriptomic responses.

Here, we compare *P. calceolata* transcriptomic response to nitrate (NO<sub>3</sub>) deprivation in situ and in vitro. Aligning *Tara* Oceans metatranscriptomic reads on the genome shows differentially expressed genes in response to changing environmental conditions, especially NO<sub>3</sub> concentration. Cultivating *P. calceolata* in depleted NO<sub>3</sub> media reveals transcriptomic response to low NO<sub>3</sub>. This dual approach allows us to define a set of genes involved in acclimation to low NO<sub>3</sub> at the intersection of both methods, reducing inherent biases. Finally, we suggest that the regulation of NO<sub>3</sub> assimilation in *P. calceolata* is central to its ecological success in oligotrophic waters.

# Population dynamics of *Ostreopsis cf ovata* in Marina del Este, Alborán Sea (Granada, Spain)

De la Rosa Álamos, J. & González Castelli, E.

Laboratory Phycology. Botanical Department. Universidad Granada, España jdlarosa@ugr.es

The genus *Ostreopsis* includes species that produce massive blooms and/or synthesize toxic compounds (palitoxins) that can be transferred through marine food webs or aerosols and cause ecological, health, and socioeconomic impacts. *Ostreopsis* species are increasing their biogeographic distribution from tropical to temperate waters and causing recurrent blooms in certain coastal areas that are of emerging global concern. In recent decades, blooms of *Ostreopsis* species have increased in Mediterranean coastal areas. *Ostreopsis cf. ovata*, the most abundant and widely distributed species on the Mediterranean coasts that has developed blooms in the last ten years.

This work presents preliminary results of the population dynamics of Ostreopsis cf ovata on Ericaria selaginoides and on Halopteris scoparia in Marina del Este, Alboran Sea (Granada, Spain). Both macroalgae constitute shallow subtidal populations in said locality. Three monthly replicates were collected on each host species according to the BEDI method, which were compared with control samples. The observed results show a high spring density compared to autumn and summer with very low densities. Halopteris scoparia harbors a larger population of O. cf ovata compared to E. selaginoides.

This study aims to develop a monitoring system to provide the administration with management tools to detect proliferations of *Ostreopsis* early and avoid its negative effects on the ecosystem or health.

#### Comparing the effects of periodic and continuous thermal selection on the fitness of a coral microalgal symbiont

Scharfenstein, H.J.<sup>1,2</sup>, Peplow, L.M.<sup>2</sup>, Alvarez-Roa, C.<sup>2</sup>, Nitschke, M.R.<sup>2</sup>, Buerger, P.<sup>3</sup>, Chan, W.Y.<sup>1</sup> & van Oppen, M.J.H.<sup>1,2</sup>

<sup>1</sup>School of BioSciences, The University of Melbourne, Parkville, VIC, AUS <sup>2</sup>The Australian Institute of Marine Science, Townsville, QLD, AUS <sup>3</sup>Applied BioSciences, Macquarie University, Sydney, NSW, AUS hscharfenste@student.unimelb.edu.au

The susceptibility of reef-building corals to thermal stress is strongly dependent on their microalgal endosymbionts (dinoflagellates in the family Symbiodiniaceae). Hence, the manipulation of coral

#### MONDAY 21<sup>st</sup> August 2023 Room « Tali friz »

photosymbionts is being investigated as a tool to increase coral resilience to marine heatwaves induced by climate change. Using experimental evolution, the thermal tolerance of Symbiodiniaceae can be enhanced *in vitro* and subsequent reintroduction of heat-evolved photosymbionts into corals can boost host fitness under thermal stress.

To date, experimental evolution studies aiming to enhance thermal tolerance in Symbiodiniaceae cultures have relied on continuous exposure to elevated temperatures. Whilst this approach has been successfully applied across multiple genera, studies with other microalgae have shown that temperature profiles incorporating periodic fluctuations displayed increased strength and efficacy of selection compared with stable temperature profiles.

In this study, we compared the efficacy of periodic versus continuous thermal selection at increasing the thermal tolerance of the coral photosymbiont *Durusdinium trenchii*, a species that previously did not respond positively to continuous selection. After two years of experimental evolution, we constructed a thermal performance curve for growth, photochemical efficiency and respiration for thermally selected and wild-type lineages at temperatures ranging 21-33°C. Preliminary results suggest selection under diurnally fluctuating temperatures, rather than fluctuations spanning multiple generations, led to the greatest increase of the thermal maximum. Our study sheds light on how the periodicity of thermal stress affects coral photosymbiont fitness across their innate thermal range, and how experimental evolution strategies for enhancing the thermal tolerance of microalgal cultures can be optimised.

### Pelagic *Sargassum* events in Jamaica: provenance, morphotype abundance, and biomass composition

Machado, C.B.<sup>1</sup>, Maddix, G.-M.<sup>2</sup>, Francis, P.<sup>2</sup>, Thomas, S.-L.<sup>3</sup>, Burton J.-A.<sup>4</sup>, Langer, S.<sup>5</sup>, Larson, T.R.<sup>5</sup>, Marsh, R.<sup>6</sup>, Webber, M.<sup>2</sup> & Tonon, T.<sup>1</sup>

<sup>1</sup>Centre for Novel Agricultural Products (CNAP), Department of Biology, University of York, United Kingdom. <sup>2</sup>Centre for Marine Sciences, University of the West Indies, Mona, Kingston, Jamaica. <sup>3</sup>Discovery Bay Marine Laboratory, Jamaica.

<sup>4</sup>Port Royal Marine Laboratory, Jamaica.

<sup>5</sup>Metabolomics and Proteomics Lab, Bioscience Technology Facility, Department of Biology, University of York, United Kingdom.

<sup>6</sup>School of Ocean and Earth Science, University of Southampton Waterfront Campus, National Oceanography Centre, United Kingdom.

thierry.tonon@york.ac.uk

Pelagic sargassum species have been known for centuries in the Sargasso Sea of the North Atlantic Ocean. In 2011, a new area concentrating high biomass of these brown algae started developing in the Equatorial Atlantic Ocean. Since then, massive and recurrent sargassum influxes, corresponding to morphotypes *Sargassum natans* I, *S. natans* VIII, and *S. fluitans* VIII, have been reported in the Caribbean and West Africa. These events negatively impact coastal ecosystems and nearshore marine life, and affect public health, coastal living, tourism, fisheries, and maritime transport. Despite recent advances in the forecasting of sargassum events and in the analysis of the biomass composition, many knowledge gaps remain, including abundance of distinct morphotypes during sargassum events, trajectory of the seaweed mats prior to stranding, and influence of sample processing on biochemical and elemental composition. Analysis of samples harvested on the coasts of Jamaica between 2019 and 2021 showed that *S. fluitans* III was the most abundant morphotype among the three. No clear difference in the geographical origin of the sargassum mats was observed. Different processing techniques, i.e. sun-drying, shade-drying, and freeze-drying, showed variable impact on the composition of the biomass. For example, sun-drying increased the proportion of phenolic compounds, but had a deleterious impact

on fucoxanthin and monosaccharide content, except for mannitol, when compared to freeze-drying. Such information is important for the storage and transport of the pelagic sargassum biomass in the context of its valorisation.

### **KEYNOTE**

#### Climate shaping genetic biodiversity of polar marine forests

Serrão, E.A.<sup>1</sup>, Assis, J.<sup>1</sup>, Fragkopoulos, E.<sup>1</sup>, Almeida, S.<sup>1</sup>, Neiva. J.<sup>1</sup>, Pearson, G.A.<sup>1</sup>, Faugeron, S.<sup>2</sup>, Konar, B.<sup>3</sup>, Raimondi, P.<sup>4</sup> & Krause-Jensen, D.<sup>5</sup>

<sup>1</sup>Centre of Marine Sciences, University of Algarve, Portugal <sup>2</sup>Pontificia Universidad Catolica de Chile, Santiago, Chile <sup>3</sup>University of Alaska Fairbanks, USA <sup>4</sup>University of California Santa Cruz, USA <sup>5</sup>Aarhus University, Denmark eserrao@ualg.pt

Climate-driven range shifts that shape the geographical distribution of genetic diversity can drive different modes of speciation including divergence in allopatry or recombination at contact zones and allopolyploidy. Empirical evidence for these predictions in marine forests was discovered by analysing the geographical distribution of genetic variability and modelling species range shifts. This revealed novel recombined genomic diversity at recent expansion fronts that form contact zones, in addition to the more expected high and unique genetic diversity in ancient populations at estimated long-term persistence zones, some of them presently located in shrinking climatic refugia. Hotspots of diversity and evolution have also resulted from diversity and reticulation within lineages with clearly discernable ancestry and geographical distribution at sympatric contact zones. Phylogroup distributions compared with habitat suitability over time further demonstrate a role of cycles of isolation in disjunct persistence areas intercalated with ephemeral expansions and admixture at high-latitude contact zones. Multi-glacial cycles thus acted as a speciation pump for marine organisms otherwise exhibiting cosmopolite amphiboreal distributions.

### ORALS

### Multi-scale patterns in the structure of fouling communities associated with seaweeds in marinas

Leclerc, J.-C.<sup>1</sup>, Gonzalez, M.<sup>1</sup>, Pezy, J.-P.<sup>2</sup>, Raoux, A.<sup>2</sup>, Gonthier, E.<sup>2</sup>, Le Gall, T.<sup>1</sup>, Houbin, C.<sup>3</sup>, Broudin, C.<sup>3</sup>, Loisel, S.<sup>1</sup>, Crec'hriou, R.<sup>3</sup>, Sevin, L.<sup>1</sup>, Daguin-Thiébaut, C.<sup>1</sup>, Migné, A.<sup>1</sup>, Coudret, J.<sup>1</sup>, Davoult, D.<sup>1</sup>, Viard, F.<sup>4</sup>, Filbee-Dexter, K.<sup>5</sup>, Wernberg, T.<sup>5</sup>, Massé, C.<sup>6</sup> & Thiébaut, E.<sup>1</sup>

<sup>&</sup>lt;sup>1</sup>Sorbonne Université, CNRS, UMR 7144 AD2M, Station Biologique de Roscoff, Place Georges Teissier, 29680 Roscoff, France.

<sup>&</sup>lt;sup>2</sup>Unicaen, Laboratoire Morphodynamique Continentale et Côtière, CNRS UMR 6143 M2C, Université de Normandie, 24 rue des Tilleuls, 14000 Caen, France.

<sup>3</sup>Sorbonne Université, CNRS, FR2424, Station Biologique de Roscoff, Place Georges Teissier, 29680 Roscoff, France.
<sup>4</sup>ISEM, Université de Montpellier, CNRS, EPHE, IRD, Montpellier, France
<sup>5</sup>Institute of Marine Research IMR; His, Norway
<sup>6</sup>UMS AFB-MNHN-CNRS Patrimoine Naturel, Station marine d'Arcachon, 2 rue du Professeur Jolyet, 33120 Arcachon, France.
leclercjc@gmail.com

Marine urbanization causes important change to the marine environment. As they proliferate at sea, artificial infrastructures are colonized by novel assemblages and generate novel species interactions. In response to multiple and disentangled biotic and abiotic processes, some of those substrata confer a disproportionate advantage to opportunistic, stress-tolerant and often non-indigenous species in their colonization and dispersal (natural or assisted). While these structures pose severe risks of invasion in degraded environments, some taxa may be limited in their spread through complex suite of species interactions. Specifically interested in facilitation related with foundation species (including native kelp), we jointly characterized the diversity, biomass and community structure of multiple groups of organisms (seaweeds, sessile invertebrates and fish) at multiple scales (pontoon, port, and region) in NW France. In particular, a series of 18 marinas were sampled within 3 distinct ecoregions. A total of 192 taxa were identified in situ. Results reveal important variability at all spatial scales examined, which are related to multiple natural (biogeographic) and anthropogenic (uses, management, hull fouling) processes. Besides highlighting relevant covariations among components, the proposed protocol confers opportunities for future upscaled monitoring and examination of port biodiversity as well as with novel assembly rules taking place along changing coastlines.

### Niche divergence and Species Distribution Models to evaluate the spread of *Rugulopteryx okamurae* in Europe

Triay-Portella, R.<sup>1,2</sup>, Delva, S.<sup>3</sup>, de Clerck, O.<sup>3</sup>, Fernández, M.<sup>2</sup>, Martínez, B.<sup>4</sup>, Bernal-Ibáñez, A.<sup>2</sup>, Tuya, F.<sup>1</sup>, Assis, J.<sup>5</sup>, Thibaut, T.<sup>6</sup>, Altamirano, M.<sup>7</sup>, Román Muñoz, A.<sup>7</sup>, Haroun, R.<sup>1</sup>, Monterio, J.<sup>2</sup> & Canning-Clode, J<sup>2</sup>

<sup>1</sup>Grupo de Biodiversidad y Conservación (BIOCON), Instituto Universitario de Investigación en Acuicultura Sostenible y Ecosistemas Marinos (IU-ECOAQUA), Universidad de Las Palmas de Gran Canaria, Spain

<sup>2</sup>*MARE – Marine and Environmental Sciences Centre / ARNET - Aquatic Research Network, Agência Regional para o Desenvolvimento da Investigação, Tecnologia e Inovação (ARDITI), Portugal* 

<sup>3</sup>Departamento de Biología y Geología, Física y Química Inorgánica, Universidad Rey Juan Carlos, Móstoles, Spain

<sup>4</sup>*Phycology Research Group and Center for Molecular Phylogenetics and Evolution, Ghent University, Ghent, Belgium* 

<sup>5</sup>Centre for Marine Sciences, CCMARCIMAR, University of Algarve, Faro, Portugal

<sup>6</sup>Mediterranean Institute of Oceanography (MIO), IRD, Aix Marseille Université, CNRS, Université de Toulon, Marseille, France

<sup>7</sup>Departamento de Botánica y Fisiología Vegetal, Facultad de Ciencias, Universidad de Málaga, Málaga, España

raul.triay@ulpgc.es

Nonindigenous species are in non-equilibrium and represents an opportunity to study niche ecology. Niche representation in geographical space is challenging due to "Etonian noise" and the infringement of "equilibrium assumptions' to conduct correlative models. In native areas, species are distributed

#### MONDAY 21<sup>st</sup> August 2023 Room « Tali friz »

representing its occupied niche. Occupied niche is constrained by biological favorable interactions, suitable environments and dispersal capacity (BAM, diagram). In some scenarios, occupied niche could contain environments outside of the fundamental niche, due to source and sink theory. This means that some parts of species populations occupy unfavorable environments and populations are maintained thanks to recruitment from suitable habitats.

Here we applied two approaches to evaluate the expansion and future scenarios of invasive brown algae *Rugulopteryx okamurae* in Europe and worldwide. First approach was in environmental space, evaluating niche divergence of non-native populations. Second approach was the use of correlative models with native and non-native occurrences in geographic space to generate species distribution models. Finally, we used mechanistic information to reduce extrapolation risk and associated uncertainties in correlative models, generating hybrid models. Our results show the present distribution of *R. okamurae* and argue, from theoretical and up to date modeling approaches, the future scenarios of one of the most dangerous invasive species in Europe.

### The last remaining populations of *Gongolaria barbata* along the Istrian Coast (northern Adriatic)

Iveša, L., Bilajac, A., Gljušćić, E. & Smith, S.

Laboratory for the ecology of benthos, Ruđer Bošković Institute, Center for Marine Research Rovinj, Croatia \*ivesa@cim.irb.hr

Gongolaria barbata is a fucalean species that formed extensive forests along the west Istrian Coast in the northern Adriatic between 2003 and 2013, most commonly in 0.5 - 2 m depth. Unfortunately, a decline of this species has been observed since 2016, leading to its local extinction over a short time period. Surveys were conducted in 2021 and 2022 to estimate the current distribution and abundance of *G. barbata* along the western Istrian coast and our surveys identified that remaining populations are present across three distinct habitat types. We observed *G. barbata* in only a few shallow subtidal locations, a single lagoonal habitat in southern Istria and located individuals within rockpools interspersed along the coastline. The lagoon and rockpool habitats appeared to offer the most favorable conditions for *G. barbata*, acting as potential refuges despite extreme environmental conditions. In contrast, shallow water populations of *G. barbata* discovered at or near the localities of formerly known forests were sparse. The morphological characteristics of *G. barbata* showed significant variation between habitats, with more complex thalli structures in the lagoon, which displayed two morphologically distinct forms, attached and detached.

To ensure the successful conservation and restoration of the remaining G. barbata along the Istrian coast, it is essential to study their population dynamics, recruitment patterns and the effects of herbivores. This knowledge will allow us to identify important donor populations that can be used for *in situ* restoration in areas of the northern Adriatic that have been affected by fragmentation and habitat degradation.

### Species loss and long-term decline in taxonomic diversity of macroalgae in the northern Adriatic Sea over the last six decades

Falace, A.<sup>1</sup>, Bevilacqua, S.<sup>1</sup>, Kaleb, S.<sup>1</sup> & Alongi, G.<sup>2</sup>

<sup>1</sup>Department of Life Sciences- University of Trieste

<sup>2</sup>Department of Biological, geological and Environmental Sciences – University of Catania falace@units.it

The northern Adriatic is a shallow, semi-enclosed basin characterized by oceanographic features that differ from the rest of the Mediterranean Sea. Studies over the last decades show that the composition of algal vegetation has changed significantly due to land reclamation, tourism, habitat loss, pollution and climatic changes.

The analysis of the Average Taxonomic Distinctness highlighted a significant contraction of the taxonomic breadth of macroalgal species pools in the region coupled with a loss of about 20-30% species in the last three decades.

The most obvious change is a remarkable loss of the formerly predominant fucoid brown algae, whose distribution and abundance has declined over the last 50-60 years. *Fucus virsoides*, the only endemic Mediterranean *Fucus* species, has suffered the most dramatic decline and is now restricted to a few sites. The current scenario of the vegetation of the northern Adriatic has therefore completely changed the physiognomy and *Cystoseira s.l.* species occur in reduced patches restricted to a few undisturbed sites. These communities have been replaced by assemblages of perennial turf-like mats of Gelidiales and *Symphyocladiella/Xiphosiphonia*, which are well adapted to environmental disturbance and spread in

stressful habitats associated mainly with sediment movement and deposition. The absence of macroalgae at some sites or the predominance of taxa with high tolerance or ephemeral species (Rhodophyta, especially Ceramiales; Ectocarpales among the Ochrophyta; Cladophorales and Ulvales among the Chlorophyta) is mainly due to pressure from herbivores, lower light penetration, and high sedimentation rates, as reported in other areas of the Mediterranean.

### SYMPOSIUM 15 "Algae as producers of valuable compounds"

#### **KEYNOTE**

#### Recent advances in sustainable applications of Seaweeds

Hanelt, D., Abomohra, A.

Aquatic Ecophysiology and Phycology, IPM, Uni. Hamburg, Germany dieter.hanelt@uni-hamburg.de

Seaweeds have been used in various pharmaceutical, chemical and food applications. In addition, seaweed cultivation is helpful for wastewater treatment. Aquaculture farms and industrial effluents around the world result in profound pollution and environmental degradation, which is considered as a serious problem. Discharged water treatment is an effective way to reduce pollution, however, most of factories and aquaculture farms do not perform such *in-situ* treatment systems.

Seaweeds for biological treatment of wastewater take up nitrate and ammonia, which are the prevalent nitrogen compounds in domestic/urban sewage, agricultural and industrial water effluents. *Gracilaria verrucosa*, for example, can remove 91.4% nitrate, 94.5% nitrite, 100% phosphate, and 99.3% ammonia, with simultaneous enhancement of dissolved oxygen and also highly absorb heavy metals and phenols from industrial wastewater streams. Algal biomass can be converted into different forms of bioenergy by thermochemical conversion, anaerobic digestion, fermentation and lipid transesterification. Our different projects for sustainable applications of seaweeds including wastewater treatment, bioplastic, textile fibers and biofuels will be presented.

Benefits of utilization of seaweeds are their abundance, fast growth rates, low input requirements, and environmental sustainability. Compared to terrestrial plants, the annual biomass productivity of seaweeds is much higher and compared to microalgae easier to maintain and harvest. However, challenges such as the high production cost, as well as limited market opportunities, need to be addressed to fully maximize the potential of seaweed-based products. Integrated routes and biorefinery could enhance the economic feasibility and the variation of the end products.

### ORALS

### Precision aquaculture drone mapping of the spatial distribution of *Kappaphycus alvarezii* biomass and carrageenan

Alevizos, E.<sup>1</sup>, Oiry, S.<sup>1</sup>, Nurdin, N.<sup>2,3</sup> & Barillé, L.<sup>1</sup>

<sup>1</sup>Institut des Substances et Organismes de la Mer, ISOMer, Nantes Université, UR 2160, F-44000 Nantes, France.

<sup>2</sup>Marine Science Department, Marine Science and Fisheries Faculty, Hasanuddin University, Makassar, 90245, Indonesia

<sup>3</sup>Research and Development Center for Marine, Coast, and Small Island, Hasanuddin University, Jl. Perintis Kemerdekaan km.10, Makassar, 90245, Indonesia oirysimon@gmail.com This study aims to apply drone technology for high-precision monitoring of the biomass and carrageenan content of the macroalgae Kappaphycus alvarezii during cultivation cycles in South Sulawesi (Indonesia). Environmental stressors, such as non-optimal temperature, low salinity, and poor water quality, can negatively impact the growth and survival of this species, resulting in reduced yields. Therefore, careful monitoring and control of environmental factors are required to optimize biomass production and carrageenan content. To our knowledge, there has been no synoptic mapping yet of Kappaphycus cultures at a relevant spatial resolution that would allow the detection of the seaweed attached to farming systems such as floating ropes. There is a striking contrast with agriculture, where drones have been increasingly used to monitor crops, gather data, and make better decisions about managing the fields. In this work, a drone-mounted multispectral camera was used for acquiring very high spatial resolution images of macroalgae attached to longlines at regular time intervals along culture cycles. Additional in situ measurements of individual macroalgae fresh weight, biometry, and carrageenan content were used to obtain relationships for scaling up to drone images. Abiotic variables were also collected for each date to describe the environmental conditions. An extensive set of multispectral drone images was processed, combining geospatial and machine learning techniques to produce spatial distribution maps of Kappaphycus fresh biomass and carrageenan biomass at the scale of floating ropes.

### Effect of the Instant Controlled Pressure Drop (DIC) Technology for extraction of seaweed compounds

Pliego-Cortés, H.<sup>1,2</sup>, Boy, V.<sup>1</sup>, Lanoisellé, J-L.<sup>1</sup> & Bourgougnon, N.<sup>2</sup>

<sup>1</sup>*IRDL, UMR CNRS 6027, F-56300 Pontivy* <sup>2</sup>*LBCM, EMR CNR>S 6076, F-56000 Vannes* hugo-skyol.pliego-cortes@univ-ubs.fr

The Instant Controlled Pressure Drop technology (DIC), is a thermo-mechanical treatment based on a short-time heat treatment followed by a controlled abrupt pressure drop, allowing the expansion of the cells in the matrix of biological products, which could represent a potential application to aid the extraction processes of seaweed compounds.

The objective of this research is to evaluate the effects of the DIC technology on the extraction of the seaweed compounds. In a first-stage, the study was conducted on the proliferative brown seaweed *Sargassum muticum*, the influence of the steam-pressure (1 and 7 bar) and treatment time (20 and 90 second) of DIC, was evaluated on fresh and dry biomass. Fresh biomass of *S. muticum* collected in Brittany, France, was cleaned and thalli were cut into three and 10 cm length, one sample of each length was Air Impingement-dried (AID) before applied DIC treatments. The DIC treatments improved (p<0.05) the extraction compared to the control (without DIC treatment) on neutral sugar (15.6 vs 7.9% dm), sulfate groups (1.7 vs 1.18% dm) and protein (19.4 vs 15.5% dm) at 90s and 1 bar of DIC, on AID thalli of 10 cm length. By applying 20s of DIC, an increase on carotenoids: fucoxanthin, zeaxanthin and b-carotene (0.8 vs 0.5% dm) and total phenolic content (1.9 vs 1.1% dm) was observed. An increase on fucoidan (7.4 vs 6.1% dm) and alginate salts (17.4 vs 15.1% dm) was observed at 1bar using 20s and 90s of DIC. Further assays are underway using *Sargassum* from the Caribbean and the green seaweed *Ulva sp*.

### Extraction of soluble proteins by bead milling from *Tetraselmis chui* in two different physiological states

Mear, H.<sup>1,2</sup>, Gillon, P.<sup>2</sup>, Gifuni, I.<sup>1</sup>, Lavenant, L.<sup>2</sup>, Poidevin, A.<sup>2</sup> & Couallier E.<sup>2</sup>

<sup>1</sup>Algosource, France <sup>2</sup>Nantes Université, Oniris, CNRS, GEPEA, UMR 6144, France mear.hugo@laposte.net

*Tetraselmis* is an interesting microalga for food products because of its high protein content and surface productivity as well as the possibility to scale-up the culture easily. However, this specie is yet understudied compared to other algae such as *Chlorella*. Different batches of *Tetraselmis chui* were received from the same supplier, with different morphological properties, probably associated with a difference in physiology.

The batches were characterized biochemically and the rupturability was tested to characterize their behaviour to cell disruption, with one batch more fragile than the other. The bead milling of the two batches also showed striking differences in terms of cell disruption kinetic, as well as in dry matter repartition after centrifugation and in protein recovery yields within the supernatant. Nearly no differences in protein yield were observed after bead milling in comparison to freeze-thawing for the resistant batch, with a final yield of 11% of the initial proteins. However, a clear protein release was observed for the fragile batch along the milling residence time, with a final protein yield of 32%. The electrophoresis of the supernatants as a function of the milling showed very different protein profiles. The results underlined that a difference in physiology, which may come from a different life cycle stage or a different cell cycle stage of *Tetraselmis chui* had a significant impact on the following biorefinery, affecting directly the cell disruption and the protein recovery yields. The valorization of the whole microalgae thus necessitates the finest coupling between upstream and downstream.

### Zygnematophyceae and their complex phenolic compounds – a biochemical strategy preceding the flavonoids of land plants?

Remias, D.<sup>1</sup>& Procházková, L.<sup>2</sup>

<sup>1</sup>Paris Lodron University of Salzburg, Department of Environment & Biodiversity, Austria <sup>2</sup>Charles University, Faculty of Science, Department of Ecology, Prague, Czech Republic daniel.remias@plus.ac.at

By molecular means, the conjugating green algae (Zygnematophyceae) have been acknowledged for more than one decade representing the closest existing group to the land plants. This stays in contrast to their simple morphology, which is currently discussed as a possible secondary reduction. Nonetheless, events of horizontal gene transfer apparently enhanced their physiological capabilities, and consequently they can dominate in exposed habitats like peat bogs, aeroterrestrial surfaces (soil, rocks, mosses, tree bark), shallow puddles or even glacial ice surfaces. In terms of cytology and metabolism, many species possess vacuoles with abundant glycosylated phenolic compounds, some of them causing a colourful secondary pigmentation. This has been particularly shown for zygospore stages. In many cases, the structure of these water-soluble compounds is not elucidated yet, not only due to analytical constraints. Their biosynthesis is likely based on gallic acid glycosides, which was found in all investigated species so far.

The presence of complex (poly-) phenols in the Spirogyrales, Zygnematales, Serritaeniales will be demonstrated, and it seems that they are chemotaxonomically specific for each order. Furthermore,

certain phenolic represent unusual, organic metal-complexes, thus enhancing the band width of spectral absorbance.

The ecological role of these compounds as putative repellents against grazers or as UV/VIS protectants will be addressed. The distinctive phenolics of conjugating green algae have similar features like the flavonoids of "higher plants". The question if either parallel evolution took place due to habitat similarities by chance, or whether we are confronted with an early, preceding biochemical stage of development, remains open.

#### Purification of mycosporin-like amino acids by centrifugal liquid chromatography for improved structural and biological characterisation

Rieusset, L.<sup>1</sup>, Michel, T.<sup>2</sup>, Simmler, C.<sup>3</sup>, Greff, S.<sup>3</sup>, Boulho, R.<sup>2</sup>, Hardouin, K.<sup>1</sup>, Bourgougnon, N.<sup>1</sup>, Audo, G.<sup>2</sup> & Bedoux, G.<sup>1</sup>

<sup>1</sup>Laboratoire de Biotechnologie et Chimie Marine, Université Bretagne Sud, CNRS - EMR 6076, France <sup>2</sup>Gilson Purification SAS, France

<sup>3</sup>Institut Méditerranéen de Biodiversité et d'Ecologie marine et continentale, UMR 7263, CNRS, IRD, Université d'Aix Marseille, Université d'Avignon, Station Marine d'Endoume, France laura.rieusset@univ-ubs.fr

Mycosporin-like amino acids (MAAs) are a family of intracellular compounds formed via the shikimate pathway. These small molecules are produced by a wide variety of marine organisms including lichens, corals, fungi, algae and cyanobacteria. They absorb a very large proportion of UV rays and allow marine organisms to protect themselves against UV and oxidative stress.

MAAs are of growing interest for use in eco-friendly sunscreen products, but also in chemical ecology to better understand their roles in marine organisms' physiology. Nevertheless, the main limitation in studying MAAs is the difficulty in obtaining sufficient quantities of pure products to characterise their chemical structure and assess their biological activity. This project, with the company Gilson Purification, aims to develop a MAAs purification strategy from red algae extracts using a centrifugal partition chromatography (CPC) approach.

This approach, carried out on a *Porphyra sp.* extract, allowed us to highlight new MAAs. We also obtained MAA fractions highly enriched in the Porphyra 334, that allow advanced NMR analysis. The results of this work will allow the preparation of MAAs that can be used as analytical standards by manufacturers. They will also allow us to further characterise the biological activity of these compounds.

#### Application of algal technology to convert waste nutrients produced from the anaerobic digestion of farm waste and brewery process to produce biomass for feed application

Silkina, A., Tang, K. & Emran, M.

Swansea University, Singleton Park SA2 8PP, Swansea, Wales (UK) \*a.silkina@swansea.ac.uk

Agricultural and food industry (brewery) waste treatment is one of the key elements of environmental impact with direct effects on the economy and society. Algal technology is designed to enable effective recycling and valorisation of water nutrients including carbon, nitrogen, and phosphorus. The integrated

evaluation and optimisation of the sustainability of an algal biorefinery including mass and energy balances, carbon, water and nutrient use and impact analysis was assessed. A biorefinery approach of waste remediation using algal cultivation was developed at Swansea University, focusing on nutrient recovery via algal biomass in pilot facilities. Mass cultivation (up to 1500 L) was developed with a 99% nitrogen and phosphorus uptake by microalgal cultures. Cultivation in batch mode was followed by semi-continuous and continuous mode. *Nannochloropsis oceanica* (marine species) and *Scenedesmus obliquus* (freshwater species) were used as a biological model and grown on nutrients waste sources. The obtained biomass was rich in nutrient content, especially for the protein and fatty acids composition. The algal biomass was used as component of animal feed nutrition and for the extraction of valuable compounds. Conclusions were drawn on the high potential of algal biotechnology for waste remediation and nutrient recovery in the context of circular bioeconomy, despite the need for further development and scalable applications of this new technology.

#### **KEYNOTE**

### Exploring the Bioactive Potential of Microalgae: Sustainable Solutions for Health and Environment

#### Khozin-Goldberg, I.

Microalgal Biotechnology Laboratory, The French Associates Institute for Agriculture and Biotechnology of Drylands, Jacob Blaustein Institutes for Desert Research, Ben-Gurion University of the Negev, Midreshet Ben-Gurion, 8499000, Israel khozin@bgu.ac.il

Microalgae represent a valuable source of bioactive lipids, encompassing a wide range of fatty acids, their derivatives, and complex lipids. Cultivated photosynthetic microalgae offer a sustainable and renewable supply of polyunsaturated fatty acids (PUFA), including  $\omega$ -3 LCPUFA and  $\omega$ -6 LC-PUFA, which possess health-beneficial properties. The evaluation and exploration of the diversity of microalgae is pivotal for discovering novel bioactive compounds and advancing their use as feed and food ingredients. We explore the diversity of microalgae, producing LC-PUFA, by improving the stress resilience of known industrialized strains and domesticating novel strains.

The research conducted in collaboration with the Fish Health Laboratory explores the anti-inflammatory, immunomodulatory and antiparasitic potential of microalgae, focusing on LC-PUFA-producing species. Our recent studies have highlighted the multifunctional potential of microalgae as sustainable alternatives to key fish feed ingredients, fish meal and fish oil. In addition, they have shown promise as therapeutants for important fish parasitic diseases, with no or little treatment solutions, and as agents to modulate the microbiome and reduce gut inflammation in a zebrafish model of inflammatory bowel disease (IBD). Remarkably, extracts and preparation derived from the diatom *Phaeodactylum tricornutum* have exhibited efficacy against monogenean (flatworms) parasites in fish as well as antibacterial properties. Moreover, we have demonstrated the utilization of residue material from the commercial *P. tricornutum* production as a sustainable and cost-effective antiparasitic treatment.

Further research and development of such applications holds promise for enhancing human and animal health while promoting environmental sustainability.

#### ORALS

### Perspective of microalgae as a bioplatform for the production of inorganic polyphosphates

Solovchenko, A.E., Gorelova, O.A., Selyakh, I.O., Semenova, L.R., Baulina, O.I., Chivkunova O.B., Vasilieva, S.G., Scherbakov, P.N., Lukyanov, A.A., Zaytsev, P.A. & Lobakova, E.S.

Lomonosov Moscow State University, Russia solovchenko@mail.bio.msu.ru

Inorganic polyphosphates (PolyP) have a plethora of potential applications in fine and bulk chemistry and medicine as food additives, antiviral and antimicrobial agents, tissue engineering scaffolds, and environment friendly slow-release phosphorus biofertilizer. A major setback for the PolyP-(bio)technologies is the difficulty of chemical synthesis of longer-chain PolyP although short-chain PolyP are mass-produced.

During acclimation to volatile phosphorus availability in the environment, microalgae have developed capacity of efficient PolyP biosynthesis integral to their mechanisms of luxury phosphorus uptake. In microalgae, PolyP serve as a phosphorus and energy depot and a buffer for inorganic phosphate ( $P_i$ ) taken up by the cell. Cycling  $P_i$  supply to the culture to trigger the luxury uptake, it is possible to obtain microalgal biomass with a very high phosphorus content (up to several percent of cell dry weight) with a significant part of the internal phosphorus accumulated in the form of PolyP. At the same time, uncontrolled build-up of short-chain PolyP can be toxic to microalgal cell and hence deteriorative for the culture performance.

Microalgae can efficiently remove phosphorus from different type of waste- and sidestreams. Therefore, microalgal biotechnologies are advantageous for increasing the sustainability of the usage of finite, non-renewable mineral phosphate resources and protecting the environment for phosphorus pollution.

Possible application of microalgae a bioplatform for green biosynthesis of long-chain natural PolyP and related bioproducts is discussed along with its advantages and potential bottlenecks. Special attention is paid to knowledge-based strategy of parametric control of phosphorus uptake and PolyP biosynthesis by microalgal cells.

### High-Value Metabolite Profiling and Bioactivity Potential of Four Microalgae from Distinct Lineages

Herbert, H., Parkes, R., Barone, M.E., Campion, E. & Touzet, N.

School of Science; Department of Environmental Science; Centre of Environmental Research, Sustainability, and Innovation; Atlantic Technological University; Sligo; Ireland Helen.Herbert@research.atu.ie

Microalgae can synthesise high-value metabolites with applications in the nutraceutical, cosmetic or pharmaceutical sectors. For instance, compounds such as phycobiliproteins, polyphenolics, carotenoid pigments or fatty acids have demonstrated anticancer, antimicrobial, antioxidant or anti-inflammatory activities. Here, microalgae species from different lineages (diatom, dinoflagellate, chlorophyte and glaucophyte) were investigated for their content in high-value metabolites (pigments and fatty acids) as well as their bioactivity potential (antioxidant and cytotoxic activities).

HPLC-UV and spectrophotometry analyses showed a range of valuable pigments in the extracts, which included fucoxanthin (*Phaeodactylum tricornutum*), peridinin (*Amphidinium carterae*), lutein (*Tetraselmis chuii*) or phycobiliproteins (*Cyanophora paradoxa*). GC-MS analysis allowed fatty acid profiling, with promising levels of the longchain polyunsaturated fatty acids EPA (range from 11% (*A. carterae*), 14% (*P. tricornutum*), 17% (*T. chuii*) to 24% (*C. paradoxa*) and DHA (up to 8% in *Amphidinium carterae*). The antioxidant capacity of the extracts was evaluated using the FC and TEAC assays and was greater for *A. carterae* (112 and 48 µmol Trolox eq/g DW, respectively). In addition, cytotoxicity analysis against human lung and breast cancer cell lines (A549 and MCF7) showed that extracts of *C. paradoxa*, *T. chuii* and *P. tricornutum* tended to enhance cell viability while methanolic extracts of *A. carterae* showed significant cellular death, probably due to amphidinols.

Considering the increasing drive for valorising natural bioresources and the sustainability of ongoing circular economy initiatives, microalgae such as *C. paradoxa*, *T. chuii*, *P. tricornutum* or *A. carterae* offer attributes making them particularly well suited for further industrial exploitation and the biorefining of high-value bioactive compounds.

## Engineering of the recently-discovered *Synechococcus* PCC 11901, a fast-growing cyanobacteria, for the synthesis of high added-value carotenoids

Betterle, N., Gasparotto, E., Bellamoli, F. & Ballottari, M

SOLE Lab, University of Verona, Italy \*nico.betterle@univr.it

The production of added-value molecules in microalgae is limited by their growth performances in industrial systems. *Synechococcus* PCC 11901 (*Syn11901*), recently isolated (Włodarczyk, 2020), showed exceptional properties suitable for large-scale cultivation. In fact, this microalga, using light and  $CO_2$ , a) efficiently accumulates biomass, b) has a rapid duplication rate, c) grows in salty soils and d) is genetically manipulable.

This work focused on the engineering of *Syn11901* to produce astaxanthin, a valuable nonnative ketocarotenoid. Specifically, heterologous  $\beta$ -ketolase from *Chlamydomonas reinhardtii* (Perozeni, 2020) and  $\beta$ -hydroxylase from *Brevundimonas* sp.SD-212 (Menin, 2020) genes were constitutively expressed. Transformed cells (BC) efficiently accumulated astaxanthin (~90% of total carotenoids) during photoautotrophic growth. Moreover, BC cells grew faster than WT cells in the presence of highlight and continuous bubbling with CO<sub>2</sub>, possibly because of the photoprotective activity exerted by astaxanthin. In the stationary phase, the investigated lines showed a comparable biomass accumulation, reaching ~4g/L dcw upon only 4 days of growth in a photobioreactor. BC cells accumulated ~0.5g of astaxanthin per Kg of biomass produced.

The synthesis of astaxanthin caused a rearrangement in the composition of thylakoidal complexes. In fact, sucrose gradient fractionation showed that accumulation of both trimeric Photosystem I and dimeric Photosystem II was impacted in BC cells compared with WT cells. It is noteworthy that this phenomenon did not impair photoautotrophic growth of the BC transformants.

In conclusion, the engineered *Syn11901* succeeded in accumulating astaxanthin, without impairing cell growth rate, making this fast-growing cyanobacterium an ideal platform for the industrial photoautotrophic synthesis of astaxanthin.

### Elucidation of phlorotannins with high antioxidant activity in Australian and French fucoids (Phaeophyceae, Fucales)

Lam Kim, F.<sup>1,2</sup>, Stiger-Pouvreau, V.<sup>2</sup>, Cérantola, S.<sup>3</sup>, Kervarec, N.<sup>4</sup>, Conlan, X.<sup>5</sup>, Barrow, C.J.<sup>5</sup>, Biancacci, C.<sup>1,6</sup> & Bellgrove, A.<sup>1</sup>

<sup>1</sup>Deakin University, School of Life and Environmental Sciences, Centre for Marine Science, Warrnambool Campus, Princes highway, Warrnambool, Victoria 3280, Australia

<sup>2</sup>Univ Brest, CNRS, IRD, Ifremer, LEMAR, F-29280, Plouzane, France

<sup>3</sup>Service RMN-RPE, SGPLAT, Université de Bretagne Occidentale, UFR Sciences et Techniques, F-29200, Brest, France

<sup>4</sup>Service Spectrométrie de masse, SGPLAT, Université de Bretagne Occidentale, UFR Sciences et Techniques, F-29200, Brest, France

<sup>5</sup>Deakin University, Centre for Sustainable Bioproducts, Waurn Ponds Campus, 75 Pigdons Road, Geelong, Victoria 3220, Australia

<sup>6</sup>Cawthron Institute, 139 Gen Rd, Glenduan, Nelson 7071, New Zealand (current affiliation) fmlamkim@deakin.edu.au

Phlorotannins are phenolic compounds in brown seaweeds with primary roles in ecological defence. Their antioxidant properties make them particularly interesting for food, cosmeceutical and pharmaceutical applications. However, phlorotannins are difficult to characterise due to their molecular size and complexity, and potential linkages with other molecules, such as terpenes, proteins and cell-wall polysaccharides. Phlorotannins from endemic Australian fucoids have rarely been described, despite recent studies highlighting high concentrations in some intertidal species.

We compared the phlorotannins from four Australian fucoids of commercial interest with those from five well-studied and/or commercialised French fucoids. Purified phlorotannins extracts were analysed by <sup>1</sup>H-NMR spectroscopy to confirm the presence of phlorotannins at sufficient purity in the extracts, and then analysed by 2D-NMR spectroscopy to identify the type of linkages and associated classes of phlorotannins. Tandem mass spectrometry with Electrospray Ionising (ESI) was performed to obtain the mass of the molecules and characterisation of the fragment ions (TOF-MS-MS-ES+). The antioxidant activity was assessed using the DPPH, FRAP and ORAC assays.

Results showed the potential complexity and species specificity of the compounds. Different classes of phlorotannins were identified via mass spectrometry, comprising polymers with up to seven units of phloroglucinol. In addition, high antioxidant activity was found in all species in comparison with positive controls, and especially for the Australian species *Cystophora torulosa* and *Hormosira banksii*. This study advances our understanding of the structure and complexity of brown algal phlorotannins, which will aid in understanding their ecological significance and commercial potential.

### Omic approaches for the identification of biosynthetic pathways for bioactive compounds in the diatom *Thalassiosira rotula*

Di Costanzo, F., Di Dato, V. & Romano, G.

Ecosustainable Marine Biotechnology Department, Stazione Zoologica Anton Dohrn Napoli, Via Ammiraglio Ferdinando Acton 55, Giardini Molosiglio, 80133, Napoli, Italy federica.dicostanzo@szn.it

Diatoms are eukaryotic unicellular microorganisms widespread in all environments and responsible for about 20 % of the primary productivity on Earth. The evolutionary success of these organisms is greatly due to their metabolic plasticity that allows them to survive in different and, sometimes, challenging

#### MONDAY 21<sup>st</sup> August 2023 Room « Tali melen »

conditions of life through the production of a variety of bioactive molecules for defence, communication and adaptation to environmental changes. Some of these molecules also demonstrated to possess pharmaceutical, industrial and biotechnological applications.

To further explore the biotechnological potential of *Thalassiosira rotula*, a centric-chain forming diatom collected in the Gulf of Naples, we sequenced and analysed its transcriptome. We identified several pathways involved in the production of bioactive molecules, i.e. secologanin, polyketides, prostaglandins (PGs) and phytosterols. Chemical analysis revealed the production of PGs and the phytosterol 24-methylene cholesterol, confirming the functionality of the related biosynthetic pathways. We also studied *T. rotula*-associated bacteria for the production of bioactive secondary metabolites revealing the existence of three novel bacteria species, which genomes host interesting biosynthetic gene clusters. Of particular relevance is a mixed non-ribosomal peptide synthetase/type 1 polyketide synthase pathway (NRPS/T1PKS), potentially responsible for the production of a class of molecules with a wide range of bioactivities.

Altogether, our results support the relevance of the cosmopolitan species *T. rotula* and its associated bacterial community for future biotechnological applications.

### A Biorefinery Approach to Extract Multiple Bioactive Compounds from the diatom *Staurosirella Pinnata* for Drug Discovery

Savio, S.<sup>1</sup>, Rodolfo, C.<sup>2</sup> & Congestri, R.<sup>1</sup>

<sup>1</sup>Laboratory of Biology of Algae, Department of Biology, University of Rome Tor Vergata, Italy <sup>2</sup>Laboratory of Cell Biology, Department of Biology, University of Rome Tor Vergata, Italy saverio.savio@gmail.com

Diatoms produce various bioactive compounds that make these microalgae a promising platform for drug discovery within the pharmaceutical field. However, there are still several challenges, such as bioactives identification, their selectivity towards target cells, and their mechanism of action.

Here, we demonstrate the huge potential of the field-isolated benthic diatom *Staurosirella pinnata* (Ehrenberg) D.M. Williams & Round, by applying a biorefinery protocol allowing the sequential extraction of bioproducts, their purification and bioactivity testing on a set of human cell lines, and their identification using metabolomic approaches.

The hydrophilic extract displayed remarkable selectivity towards melanoma cells (80% of cell death), while leaving normal keratinocytes unharmed. This cell-type selectivity is a crucial factor for the successful development of anti-cancer drugs based on diatom metabolites. Extract composition was assessed by further fractionation, leading to the identification of different apocarotenoids and rare oxylipins.

We also obtained a lipidic extract, containing essential FAs for human health, such as hexadecenoic (31.02%), palmitoleic (25.95%), and eicosapentaenoic (10.58%) acids. We tested the bioactivity potential on adipocyte cells, in terms of lipolysis and thermogenesis pathways activation, which represent novel pharmaceutical targets for the treatment of the obesity and diabetes. Our results suggest this fraction as able to improve adipocyte mitochondria thermogenic activity, providing new evidence towards the development of diatom FAs-based dietary supplements.

Our investigations on *S. pinnata*-derived metabolites mark a significant step forward in diatom-based drug discovery, shedding light for the first time on their potential application in the pharmaceutical field.

#### A tri-continental survey on the inclusion of macroalgae in the diet

#### MONDAY 21<sup>st</sup> August 2023 Room « Tali melen »

Goecke, F.<sup>1</sup>, Aakre, I.<sup>2</sup>, Angarita, L.<sup>3</sup>, Li, N.<sup>4</sup>, Li, X.D.<sup>5</sup>, Escobar, M.C<sup>3</sup>, Cisternas, S.<sup>3</sup>, Wang, L.Z.<sup>4</sup>, Pang, S.J.<sup>5</sup> & Ergon, Å.<sup>1</sup>

<sup>1</sup>Department of Plant Sciences, Faculty of Biosciences, Norwegian University of Life Sciences, Ås, Norway <sup>2</sup>Department of Marine Toxicology, Institute of Marine Research, Bergen, Norway <sup>3</sup>Escuela de Nutrición y Dietética, Facultad de Medicina, Universidad Nacional Andres Bello, Concepción, Chile

<sup>4</sup>Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Qingdao, China <sup>5</sup>Institute of Oceanology, Chinese Academy of Sciences, Qingdao, China franz.goecke@nmbu.no

Macroalgae has been an important dietary component in many parts of the world for centuries, especially in Asia. In recent years, there is a combination of factors enhancing the use of macroalgae as food in the global market. Since macroalgae as a commercially available food are new in many countries, little is known regarding its consumption. There is a demand for data on intake to evaluate consumer exposure and food safety of such products.

In this tri-continental survey we included three distant countries, each known macroalgae producers with a long coast: Chile, China, and Norway. Our objective was to compare current uses of macroalgae as food, in a convenient sample dominated by adult male and female students. A macroalgae specific food frequency questionnaire with a 4-week recall period was used to assess intake frequencies, species, and product types.

585 respondents answered the survey. The vast majority considered macroalgae as attractive foods for its flavor and nutritional benefits. Potential dangers were much less known. In Chile and China, tradition was important in terms of consumption of macroalgae, while food novelty seemed to be a major factor in Norway. However, all three countries consumed a similar number of species (17-19) and products containing macroalgae (18). Brown macroalgae were more frequently consumed in Chile and China, but in Norway red macroalgae were preferred. Knowing what we consume, and how often, is necessary for safety evaluations and development of future food regulations.

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#### Mining Porphyridium cruentum biodiversity for high value products

Ross, M.E.<sup>1,2</sup>, Saint-Sorny, M.<sup>3</sup>, Stanley, M.S.<sup>1,2</sup>, Persillon, C.<sup>4</sup>, Bonzom-Audiffrin, C.<sup>4</sup>, Compadre, A.<sup>3,5</sup>, Fleury, G.<sup>3</sup>, Dubreuil, C.<sup>3,6</sup>, Perez-Fernandez, M.<sup>3,7</sup> & Sassi, J-F.<sup>3</sup>

<sup>1</sup>SAMS, Scottish Association for Marine Science, Oban, Argyll, PA37 1QA, UK
<sup>2</sup>CCAP, Culture Collection of Algae and Protozoa, SAMS, Oban, Argyll, PA37 1QA, UK
<sup>3</sup>CEA/DRT/CTREG, Centre de Cadarache, St Paul Lez Durance 13108, France
<sup>4</sup>Proteus by Seqens, 23 Rue Bossuet Z.I. de la Vigne Aux Loups, 91160 Longjumeau, France
<sup>5</sup>Atelier LUMA, Parc des Ateliers, 53 chemin des Minimes, 13200 Arles, France
<sup>6</sup>ENYO Pharma, 60 avenue Rockefeller, 69008 Lyon, France
<sup>7</sup>Universidad Loyola, CP: 41704, Dos Hermanas, Sevilla, Spain.
Michael.ross@sams.ac.uk

*Porphyridium cruentum* is a red microalga, which is rich in bioactive compounds, including phycobiliproteins, polysaccharides and carotenoids. *P. cruentum* can synthesize and accumulate high concentrations of zeaxanthin and  $\beta$ -carotene, which are important molecules for food and nutraceutical industries, which drives forward the commercial interest in natural carotenoid production

#### MONDAY 21<sup>st</sup> August 2023 Room « Tali melen »

To increase growth rates and enhance product yield, a strain improvement regime was performed on the wild type *P. cruentum* (UTEX 161). UV-C light was used as an indirect mutagenic agent; a total of 116 strains were subsequently isolated. These UV-induced mutants underwent a screening programme based upon growth rate, formation of aggregates, and exopolymeric substances (EPS) production with reference to the wild type.

The screening highlighted that 22 of the strains had a 39-83% increase in growth rate, in comparison to the wild type. These strains were selected for further biochemical characterisation. These mutants strains exhibited increases of up to 49% protein, 34% phycoerythrin, and 64% total carotenoid production. HPLC analysis indicated that the zeaxanthin to chlorophyll *a* ratio of the mutants ranged from 4.38 to 9.65, versus a mean of 8.21 found in UTEX 161. Preliminary upscaling and fractionation experiments revealed that UV-mutated strains show promise for larger-scale cultivation and implementation into a biorefinery with multiple valuable extracts as outputs.

The ABACUS project has received funding from the Bio Based Industries Joint Undertaking (BBI-JU) under grant agreement No 745668. The BBI-JU receives support from the European Union's Horizon 2020 research and innovation program and from the Bio Based Industries Consortium.

### Characterization of Emulsions Stabilized by Polysaccharide from The Red Microalga *Porphyridium* sp.

Irit, D.<sup>1</sup>, Meshulam, D.<sup>3,4</sup>, Kravtsov, A.<sup>2</sup>, Lesmes, U.<sup>3,4</sup> & Malis Arad, S.<sup>2</sup>

<sup>1</sup>Sapir Academic College, P.M. Hof Ashkelon 79165, Israel
<sup>2</sup>Dept. of Biotechnology Engineering, Ben-Gurion University of the Negev, Beer-Sheva 84105, Israel
<sup>3</sup>Dept. of Biotechnology and Food Engineering, Technion – IIT, Haifa, Israel
<sup>4</sup>Russel Berrie Nanotechnology Institute, Technion- IIT, Haifa, Israel
<sup>i</sup>ritd@sapir.ac.il

An ongoing search exists for novel, bioactive ingredients that are capable of enhancing and extending the functionality of emulsions. One area that has generated great interest is the isolation of compounds from marine sources due to their numerous beneficial health effects. This is true of marine algae which contain structurally diverse bioactive compounds. Of particular interest is the red microalga *Porphyridium* sp. whose cells are encapsulated within a sulfated polysaccharides and extracellular portions are dissolved in the growth medium. Those sulfated polysaccharides are negatively charged and can be used for a variety of applications in the cosmetic, pharmaceutical and food industries. This study investigated the use of the sulfated polysaccharides from the red microalga *Porphyridium* sp. as potential stabilizers in food emulsions.

The properties of emulsions produced through high pressure homogenization (at 117 MPa) were characterized in terms of size and electrokinetic charge based on static and dynamic laser scattering. Emulsion physical stability was further investigated through analytical centrifugation (LUMisizer, LUM GmbH), which enabled a comparative investigation of different formulations. These experiments showed that optimal emulsion formulation can be reached using 10:1 (w/w) oil:emulsifier ratio. Furthermore, we present findings regarding emulsion responsiveness to major environmental stressors (2<pH<8 and salinity 0-300 mM NaCl). Overall, the data presented highlight the potential of using functional sulfated polysaccharides from red microalgae in food applications as illustrated in this study of emulsion stabilization. This information is believed to be of importance for possible utilization of red microalgal polysaccharides as novel emulsifiers in the rational design of food emulsions and potentially in other food applications.

### Evolution of thalli and polysaccharide quality of holopelagic *Sargassum* species during their drift until their stranding

Stiger-Pouvreau, V.<sup>1</sup>, Chatelain, B.<sup>1</sup>, Kergosien, N.<sup>1,2</sup>, Deslandes, E.<sup>1</sup>, Cosquer, N.<sup>3</sup>, Dromard, C.<sup>4</sup>, Thouard, E.<sup>5</sup>, Thibaut, T.<sup>6</sup>, Changeux, T.<sup>6</sup>, Berline, L.<sup>6</sup> & Connan, S.<sup>1</sup>

<sup>1</sup>Univ Brest, CNRS, IRD, Ifremer, LEMAR, F-29280 Plouzane, France
<sup>2</sup>ALGAIA, R&D center, Saint-Lô, France
<sup>3</sup>Univ Brest, CNRS, CEMCA, F-29238 Brest, France
<sup>4</sup>BOREA, Université des Antilles, MNHN, Sorbonne Université, Université de Caen Normandie, CNRS, IRD, 97157 Pointe-à-Pitre, Guadeloupe, France
<sup>5</sup>IFREMER, Unité Biodiversité et Environnement de la Martinique, 97231 Le Robert, France
<sup>6</sup>Aix Marseille Univ, Université de Toulon, CNRS, IRD, MIO, Marseille, France
Valerie.Stiger@univ-brest.fr

Since 2011, coastal populations in the Caribbean region suffer repeatedly from sargasso strandings with catastrophic consequences for human health together with an impact on benthic fauna and flora. Pelagic *Sargassum*, with three recognized morphotypes *S. fluitans* III, *S. natans* I, *S. natans* VIII, grows offshore and forms rafts up to several hundred meters in diameter. These rafts undergo accelerated degradation as they approach shallow coastal waters. Within the ANR SAVE-C project, the aims of this study were to characterize the structure (thallus size and morphotype proportion) of *Sargassum* rafts and to follow the quality of polysaccharides along their drift and during their beaching in two Islands from French West Indies. Fucans and alginates were extracted and analysed using spectroscopic and biochemical approaches. The different analyses revealed fluctuations of both algal metabolites for each morphotype along the rafting and towards the degradation of the thalli (beaching). The fluctuations of the M/G ratios of alginates and the sulphation rates of fucans depend on the morphotype, and a particular tendency that seems common to the three morphotypes concerns the M/G ratio of alginates whose content was higher in the open sea than in beached thalli. We also found that the fragmentation rate increased for stranded *Sargassum*. Results are discussed with regard to the fragmentation of the thallus when it strands and to the potential valorization which could be recommended for this huge and seasonal biomass.

#### PLENARY

### From single cells to populations – towards understanding phytoplankton diversity and adaptation

#### Rengefors, K.

Department of Biology, Lund University, Sweden karin.rengefors@biol.lu.se

Phytoplankton inhabit most aquatic habitats on earth and are major players in biogeochemical cycles, yet we are far from understanding several fundamental aspects of their ecology and evolution. These include how their large populations and short generation times affect their ability to adapt and speciate. My research interests are especially focused on the processes underlying population differentiation and adaptation, particularly in bloom-forming species. I will present some examples of my research team's work, including population genetic patterns in the invasive freshwater species Gonyostomum semen (Raphidophyceae), rapid adaptation in the marine diatom *Skeletonema marinoi*, and intraspecific genetic and phenotypic diversity in the toxin-producing cyanobacterium Microcystis. For G. semen we wanted to investigate its population genetic structure to understand its distribution and alleged invasiveness. To do this in we utilized RAD-sequencing for genotyping and developed a single-cell method to circumvent culturing. In another projecte, our aim was investigate phytoplankton resilience to human-induced environmental change. Specifically, we studied adaptation to copper in S. marinoi populations from the Baltic Sea. To that end, we developed a strain-specific barcoding tool and set up an artificial evolution experiment in the laboratory. With the long-term goal to understand why some Microcystis produce the toxin microcystin and others not, we zoomed in on the diversity within a bloom. There we explored the diversity in microcystin variant production as well as in the microcystin gene cluster. In this lecture, I will present our main results, discuss the challenges, and potential avenues forward.

### SYMPOSIUM 02 "Biodiversity assessment and algal distribution in space and time"

#### **KEYNOTE**

### The hidden flows within species: Phytoplankton population dynamics in Arctic assemblages

Wolf, K.E.K.<sup>1,2</sup>, Hoppe, C.J.M.<sup>2</sup>, Rost, B.<sup>2,3</sup>, Schaum, E.<sup>1</sup> & John, U.<sup>2,4</sup>

<sup>1</sup>University of Hamburg, Institute for Marine Ecosystem and Fishery Science, Olbersweg 24, Hamburg, Germany

<sup>2</sup>Alfred Wegener Institut – Helmholtz-Zentrum für Polar- und Meeresforschung, Marine Biogeosciences/Chemical Ecology, Am Handelshafen 12, Bremerhaven, Germany

<sup>3</sup>University of Bremen, FB2, Bibliothekstraße 1, Bremen, Germany

<sup>4</sup>Helmholtz Institute for Functional Marine Biodiversity (HIFMB), Ammerländer Heerstraße 231, Oldenburg, Germany

klara.wolf@uni-hamburg.de

Progressing climate change and concurrent alterations of environmental conditions pose challenges of adaptation on organisms and ecosystems, especially in rapidly changing places like the Arctic. While more diverse systems are usually considered to be more resilient, biodiversity does not only describe the number of species, but can also consist of diverse individuals within a species. Especially in protists, with large census sizes and fast proliferation, intraspecific lineage sorting can be an important mechanism of plasticity and trait adjustment. For phytoplankton communities at the base of the foodweb, physiological acclimation and species shifts are frequently described, but intraspecific composition and diversity are methodologically still difficult to resolve, especially in diverse natural contexts and at temporal resolution. Therefore, our knowledge on the functioning and importance of intraspecific selection dynamics in phytoplankton is still limited. In recent years, we have developed and applied a new, high throughput methodology for phytoplankton population composition, which can make temporal and spatial population dynamics visible that were before extremely difficult to resolve. Next to experiments with natural phytoplankton communities and artificial populations under controlled settings, a time-series of Arctic spring blooms has been investigated towards the year-to year composition of species but also of intraspecific populations of a dominant diatom. Datasets emerging now thanks to such novel technologies can offer new, more comprehensive perspectives on our understanding of the mechanisms and results of microevolution and local adaptation, and can reveal formerly hidden patterns of species' strategies of persistence and development.

#### ORALS

### Synoptic observations of small-scale phytoplankton variability based on a citizen science program

Schweibold, L.<sup>1</sup>, Klein, C.<sup>1</sup>, Cadier, M.<sup>2</sup>, Sourisseau, M.<sup>2</sup>, Bihannic, K.<sup>1</sup> & Pondaven, P.<sup>1</sup>

<sup>1</sup>Laboratoire des Sciences de l'Environnement Marin (Lemar, IUEM) France <sup>2</sup>DYNamique des Ecosystèmes CÔtiers (DYNECO, Ifremer) France laura.schweibold@univ-brest.fr

Synoptic observations of small-scale (<1 km) variability in phytoplankton community composition based on in situ sampling are rare. Thanks to a citizen science program called "Objectif Plankton", such synoptic sampling of phytoplankton communities has been performed in a macrotidal environment (the bay of Brest, Brittany, France) since 2014. Using a predefined protocol, and a simplified plankton sampling kit, 17 volunteer boaters sample, simultaneously, distinct geolocated sites. Sampling is conducted thrice annually, in April, June, and September. Collected samples are brought back to the laboratory for subsequent analysis of phytoplankton community composition and other environmental variables.

In parallel, the validity of the protocol was tested; i.e. depending on the location of the sampling stations, the weather conditions, or the nature of their embarkation, volunteers may take up to 4 hours to bring the water samples back to shore. To account for potential bias due to this delay, a separate sensitivity analysis was conducted and permitted to validate the method.

Preliminary results, obtained on April 24, 2021, are presented, including measurements of several abiotic parameters (e.g., nutrient concentrations) and different methods used to characterize phytoplankton community composition: light microscopy counts, chemotaxonomy (HPLC), and flow cytometry. Despite a spring phytoplankton bloom observed in the bay during the sampling period, the data show a non-uniform composition of phytoplankton communities at a small scale, with distinct patches dominated by different taxa. These spatialization patterns are discussed with regard to the strong hydrodynamic activity due to the tides in the Bay of Brest.

### Photoacclimation in the mixotrophic ciliate *Mesodinium rubrum*: phenotypic variability and implications for red tide remote sensing

Pochic, V.<sup>1,2</sup>, Gernez, P.<sup>1</sup> & Lacour, T.<sup>2</sup>

<sup>1</sup>Nantes Université, Institut des Substances et Organismes de la Mer, ISOMer, UR 2160, F-44000 Nantes, France

<sup>2</sup>Ifremer, PHYTOX, Laboratoire PHYSALG, F-44000 Nantes, France victor.pochic@univ-nantes.fr

*Mesodinium rubrum* is a kleptoplastidic ciliate, that acquires photosynthetic capacities by sequestering the chloroplasts and nucleus of its cryptophyte prey *Teleaulax amphioxeia*. Recent studies have shown that *M. rubrum* is able to photoacclimate, despite a loss of regulation in the transcription of photosynthesis-related genes in its kleptokaryon. Blooms of *M. rubrum* cause red tides that have been observed in numerous places in the world's oceans, that can be observed by satellite remote sensing. Studying the pigments and optical properties of *M. rubrum* is a crucial step towards the development of tools for characterizing the biomass of this ciliate from high resolution satellite images.

Using cultures maintained at different irradiance levels, we observed that photoacclimation in *M. rubrum* closely ressembles that of its cryptophyte prey, prompting substantial variability in pigment composition and absorption properties depending on irradiance. Phycoeryhtrin 545 seems to play a major role for photoacclimation in *M. rubrum* and *T. amphioxeia*, with cellular concentrations three times higher in the lowest irradiance compared to the highest. Absorption cross section decreased, and specific absorption coefficients increased with irradiance intensity, at the peak absorption wavelengths of chlorophyll *a* and phycoerythrin 545. We compared the pigment ratios and absorption properties of cultures to field measurements acquired during blooms of *M. rubrum*, using data from the literature and from a field sampling conducted on the French Atlantic coast. Overall, this work provides new insights in the photophysiology of a peculiar phytoplankton, and suggests guidelines for future studies of *M. rubrum* blooms in natural environments.

### Genetic diversity and structure of *Undaria pinnatifida* populations in China and their genetic relationship with those from other countries

Shan, T.F.& Li, Y.Q.

CAS and Shandong Province Key Laboratory of Experimental Marine Biology, Institute of Oceanology, Chinese Academy of Sciences, 7 Nanhai Road, Qingdao 266071, China shantifeng@qdio.ac.cn

Undaria pinnatifida is a kelp that is extensively farmed as a marine crop in East Asia. In China it is now naturally distributed on rocky shores between the latitudes of  $28^{\circ}$  and  $40^{\circ}$  N. We used nuclear microsatellites and mitochondrial sequences to analyze the genetic diversity and structure of *U. pinnatifida* populations in China and their genetic relationship with those from other countries. We found high genetic diversity in both wild and farmed populations from China with the values of expected heterozygosity > 0.6 for microsatellites. Significant genetic differentiation was detected among geographically isolated wild populations and between wild and farmed populations. The high genetic differentiation between the farmed populations and the adjacent subtidal wild populations suggests limited genetic connection between them.

Phylogenetic analyses based on mitochondrial DNA revealed that the Chinese populations were classifiable as the Continental and Northern Japan types. All wild populations from rocky reefs in northern China were grouped with the Continental type and all farmed populations with the Northern Japan type. The results support the historical documents that China only has its native *U. pinnatifida* in southern provinces, the northern wild populations originated from Korea, and the farmed stocks were introduced from Japan. We found much lower genetic diversity in European introduced populations, especially the most recently discovered populations on Sylt in the northern Wadden Sea, indicating remarkable founder effects. Mitochondrial haplotype composition of the Sylt population is similar to that of other European populations, suggesting the latter most likely being the direct origin of the former.

### Seascape genomics of *Saccharina latissima* from the St. Lawrence estuary and gulf (Canada)

Martín-Martín R.P.<sup>1,2</sup>, Treillefort, M.<sup>1</sup>, Salvo, F.<sup>2</sup>, Valero, M.<sup>3</sup> & Dufresne, F.<sup>1</sup>

<sup>1</sup>Département de biologie, chimie et géographie, Université du Québec à Rimouski, 300, Allée des Ursulines, Rimouski, QC, G5L 3A1, Canada

<sup>2</sup>Merinov, 96, montée de Sandy Beach, bureau 2.05, Gaspé, QC, G4X 2V6, Canada

<sup>3</sup>Station Biologique de Roscoff, CS 90074, Place Georges Teissier, 29688, Roscoff cedex, France

RafaelPablo.Martin@uqar.ca

Understanding the relation of genetic and population structure with seascape attributes is essential to comprehend species dynamics, predict their response to climate change scenarios, establish conservation strategies, and define guidelines for commercial exploitation. This is particularly relevant for keystone and economically important species such as the seaweed *Saccharina latissima*. This taxon plays a significant role in providing ecosystem services in the Northern Atlantic marine flora and is one of the most important aquaculture exploited species in Western countries.

In this study, a seascape genomic perspective was applied to *S. latissima* populations along eastern Quebec coasts (St-Lawrence estuary and surrounding gulf), which has not been used for commercial seaweed species in this area before. Samples were collected from 12 points, and DArTseq markers were obtained to analyse population structure (PCoA) and test for isolation by distance (IBD) using Euclidean and in-water distances (Mantel and dbMEM analysis). Additionally, clustering techniques were employed to characterize the seascape configuration and composition of sampling sites using relevant biotic and abiotic environmental variables from the Copernicus marine datasets.

The results indicated significant IBD, and both dbMEM and PCoA identified at least three distinct genetic groups in the populations studied (St. Lawrence estuary, eastern Gaspésie and Iles de la Madeleine), which aligned with the three main groupings identified in the sampling sites according to environmental variables. These findings will facilitate better genetic variability characterization of the populations based on seascape attributes and have significant implications for conservation and exploitation guidelines for *S. latissima* populations.

### Sediment archives as time-series of millennial phytoplankton adaption

Bolius, S.<sup>1</sup>, Schmidt, A.<sup>2</sup>, Kaiser, J.<sup>1</sup>, Arz, H.W.<sup>1</sup>, Dellwig, O.<sup>1</sup>, Karsten, U.<sup>3</sup>, Epp, L.<sup>2</sup> & Kremp, A.<sup>1</sup>

<sup>1</sup>Leibniz-Institute for Baltic Sea Research Warnemünde, Germany <sup>2</sup>University of Konstanz, Germany <sup>3</sup>University of Rostock, Germany sarah.bolius@io-warnemuende.de

Climate change and human impact have led to a profound decrease in marine biodiversity and changes in species composition of phytoplankton communities in the Baltic Sea. Yet, the Baltic Sea underwent environmental changes during its Holocene history with alternating phases of warm and cold temperatures, varying salinity and nutrient availability, and additionally an increase intensity of human impact.

Some phytoplankton species are able to form resistant resting stages, which allow longterm dormancy. When resting stages settle to the sea floor after annual blooms, they accumulate in e.g. distinct sediment layers, and can serve as natural archives of phytoplankton communities and populations representing different time points in history. Thus, they offer a unique opportunity to trace changes in population structure and function through centuries and even millennials.

Here we explore the potential of phytoplankton long-term survival in a Holocene sediment core from the eastern Gotland Basin, central Baltic Sea. Germination experiments with sediment samples from different depths in the sediment core representing phases of alternating warm and cold phases of the last ca. 8000 years, were conducted in the laboratory. These resurrection experiments yielded 7 cohorts of strains of the spring-bloom diatom *Skeletonema marinoi* from around 7000 years ago until the present.

The first results of phenotyping experiments for temperature reaction norms, cellular nutrient ratios and photosynthetic activity data, and genotyping analysis, suggest higher growth for strains under higher temperatures, in accordance with their habitual ambient temperature during past warm phases.

### Phytoplankton response to marine heatwaves tendencies in the Northern Adriatic Sea

Neri, F., Garzia, A., Romagnoli, T., Accoroni, S., Memmola, F., Ubaldi, M., Coluccelli, A., Falco, P.P. & Totti, C.

Dipartimento di Scienze della Vita e dell'Ambiente, Università Politecnica delle Marche. Ancona, Italy f.neri@pm.univpm.it

In the recent decades, the Northern Adriatic Sea (NAS), one of the most productive areas of the Mediterranean Sea, faced several changes in both the trophic status and phytoplankton community structure related to anthropogenic and meteoclimatic pressures. Among the latter, marine heatwaves (MHW) are expected to have an important impact. The aim of this study was to analyze the tendencies phytoplankton biomass and abundances in relation with MHW frequency, duration and intensity during a 40-year period in the NAS.

Satellite data, sea surface temperature (1982–2022) and chl-a (1998–2022), were analyzed through MATLAB and r software. MHW event was considered when temperatures were warmer than 90<sup>th</sup> percentile for at least 5 consecutive days.

Increasing trends of MHWs frequency (p<0.001) and duration (p<0.01) were observed, with a change points (CP) in the time series tendencies in 2011 and 2001, respectively. Mean intensity showed a significant increasing trend only in some areas (e.g. Po River delta). Significant decreasing trends of chla were found in the Po River delta (p<0.001, CP in 2002), Grado Lagoon and Gulf of Trieste (p<0.001), eastern Adriatic coast and offshore areas under and above the 40 m depth (p<0.001, CP in 2002 and 2014 and p<0.01, CP in 2001, respectively).

The detailed analysis of phytoplankton communities in 2 coastal and offshore LTER stations (1988-2022) showed decreasing trend of dinoflagellates and diatoms, highlighting important effects of meteoclimatic events on phytoplankton biomass and abundances, posing a great concern about the effects on marine services and food webs.

# Exploring patterns of species diversity and species geographic distribution of epiphytic filamentous red algae (EFAs) affecting *Kappaphycus* and *Eucheuma* macroalgae farming in the Western Indian Ocean

Brakel, J.<sup>1,2,3</sup>, Ruiz Gonzalez, C.<sup>1</sup>, O'Connell<sup>1</sup>, C., Zhang, Q.Y.<sup>1</sup>, Matoju, I.<sup>4</sup>, Rusekwa, S.<sup>4</sup>, Ndawala, M.<sup>4</sup>, Msuya, F.E.<sup>4</sup>, Faisan, J.Jr.<sup>5</sup>, Brodie, J.<sup>6</sup>, Robinson, G.<sup>1</sup>, Pascal, F.<sup>7</sup>, Joanne, P.<sup>7</sup> & Gachon, C.<sup>1,8</sup>

<sup>&</sup>lt;sup>1</sup>Scottish Association for Marine Science, Scottish Marine Institute, Oban, United Kingdom

<sup>&</sup>lt;sup>2</sup>Helmholtz Institute for Functional Marine Biodiversity at the University of Oldenburg, Germany

<sup>&</sup>lt;sup>3</sup>Alfred Wegener Institute Helmholtz Center for Polar and Marine Research, Germany

<sup>&</sup>lt;sup>4</sup>Department of Botany, University of Dar es Salaam, Dar es Salaam, Tanzania

<sup>&</sup>lt;sup>5</sup>Aquaculture Department, Southeast Asian Fisheries Development Centre, Iloilo, Philippines

<sup>&</sup>lt;sup>6</sup>Natural History Museum, Science, London, United Kingdom

#### TUESDAY 22<sup>nd</sup> August 2023 Room « Tali ebrel »

<sup>7</sup>Ocean Farmers, Madagascar <sup>8</sup>Unité Molécules de Communication et Adaptation des Micro-organismes, UMR 7245, Muséum National d'Histoire Naturelle, CNRS, Paris, France janina.brakel@hifmb.de

The red algal eucheumatoid genera Kappaphycus and Eucheuma are cultivated in the tropics for their carrageenan content, reaching an annual production of 9.9 million tonnes in 2020. The first commercial scale cultivation of Kappaphycus and Eucheuma was initiated in the Philippines in the 1970s. In the Western Indian Ocean, commercial farming was initiated with cultivars imported from Southeast Asia mainly in Tanzania and Madagascar. An increasing challenge in the cultivation of Kappaphycus and *Eucheuma* in this region is the occurrence of epiphytic filamentous red algae (EFAs), including species of the red algal genera Melanothamnus, Herposiphonia, Ceramium and Centroceras. Here, we report on an investigation to study patterns of diversity and distribution of macroalgae and associated organisms of relevance in seaweed aquaculture by a high-throughput multiplexed barcoding approach. For the first-time, species identity of EFAs has been determined using molecular characterisation in this region and coupled with morphological characterisation. Using a hierarchical structured sampling design, we collected EFAs from both farmed eucheumatoids and from those growing in the wild in close proximity to the farms in Tanzania and Madagascar. The approach, combining morphological characterisation with a high-throughput molecular barcoding, will be presented and discussed in relation to species distribution and patterns of diversity of these cryptic species that are hampering Kappaphycus and Eucheuma cultivation.

### Long-term monitoring of intertidal macroalgal communities: ecological quality assessment and trajectory analysis

Bizien, A.<sup>1</sup>, Toumi, C.<sup>1</sup>, Gauthier, O.<sup>1</sup>, Sturbois, A.<sup>2</sup>, Helias, M.<sup>1</sup>, Boyé, A.<sup>3</sup>, Le Duff, M.<sup>4</sup> & Burel, T.<sup>1</sup>

<sup>1</sup>LEMAR, France <sup>2</sup>VivArmor Nature, France <sup>3</sup>Ifremer, France <sup>4</sup>UAR 3113, France anaelle.bizien@etudiant.univ-brest.fr

Following numerous oil spills, the REBENT program (Benthic Network) was established to monitor benthic habitats along the coast of Brittany. Starting in 2004, data on intertidal macroalgal communities have been gathered every three years since then. By compiling 19 years of monitoring on 10 sites, our study aims at characterizing temporal variations in the structure of macroalgal communities. The high shore communities were anticipated to show strong variations due to observed loss of vegetation cover over the years, as noted by experts.

We used Community Trajectory Analysis to quantify and compare temporal community dynamics through metric computation. On the 6 communities found in the area, *Pelvetia canaliculata* and *Fucus serratus* communities had the highest resemblance between 2004 and 2022, indicating no conspicuous long-term changes. Contrarily, the *Ascophyllum nodosum* community showed large variability, indicating an ecological drift which might respond to a perturbation. When focusing on the *A. nodosum* community, high segment lengths (*i.e.*, dissimilarity between consecutive observations) and increasing net changes (*i.e.*, dissimilarity from the initial observation) indicated strong changes in southern Brittany sites. Trough an Ecological Quality assessment, observations were compared to a cluster of reference

points, considered pristine. This new statistical method revealed regionally heterogeneous ecological statuses, consistent with already existing indicators.

This study highlights 1) the importance of trajectories analysis to understand temporal changes at stational and community levels, 2) the pertinence of statistical quality indicators to define the good health of ecosystem, and 3) suggests the method applicability to other monitoring programs (*e.g.* WFD, MSFD).

### Recent changes in rocky shore biodiversity across the NE Atlantic coast

Monteiro, C.<sup>1,2</sup>, Meneghesso, C., Arenas, F.<sup>3</sup>, Pereira, J.<sup>1,2,4</sup>, Seabra, R.<sup>1,2</sup> & Lima, F.P.<sup>1,2</sup>

<sup>1</sup>CIBIO, Research Centre in Biodiversity and Genetic Resources - InBIO Associate Laboratory, Campus of Vairão, University of Porto, Vairão, Portugal

<sup>2</sup>BIOPOLIS Program in Genomics, Biodiversity and Land Planning, CIBIO, Campus of Vairão, University of Porto, Vairão, Portugal

<sup>3</sup>CIIMAR Interdisciplinary Centre of Marine and Environmental Research of the University of Porto, Porto, Portugal

<sup>4</sup>Department of Biology, Faculty of Sciences, University of Porto, Porto, Portugal cmonteiro@cibio.up.pt

Understanding the impacts of warming in coastal ecosystems requires synthesising community responses underway within communities at the ocean basin scale. Standard and repeated surveys are key to track change over time. Here, we compare biodiversity patterns of the Atlantic rocky intertidal zone in two time periods: 2000-2005 and 2017-2019. Surveys were carried out following the same protocol: 15 50x50 cm quadrats were surveyed in the low and 10 50x50 cm quadrats in the mid-intertidal zone, where percentage cover of sessile taxa and individual counts of mobile taxa were recorded in situ. In addition, an *ad-libitum* search was done to record the abundance of taxa not covered by quadrat sampling (hard-to-reach habitats, rare species and others). Algae and animals were identified at the species level, whenever possible, in more than 160 taxa. The surveys were performed along an area spanning 24 degrees of latitude (42 locations from Ireland to Morocco), encompassing a variety of climates and their associated biodiversity patterns. We analysed biodiversity indices, functional traits and community temperature indices. A shift in dominance from coldwater and canopy-forming species to warm-water, low-lying turf mats has occurred and is prevalent across the study area. Results highlight changes in local biodiversity across the NE Atlantic intertidal and suggest that a reduction of the three-dimensional structure of its temperate reefs might be underway, with potential yet unclear repercussions for the ecosystem.

### Inferring population genetic structure from meta-transcriptomic samples

Le Gac, M.<sup>1</sup>, Mary, L.<sup>1</sup>, Metegnier, G.<sup>1</sup>, Quéré, J.<sup>1</sup>, Siano, R.<sup>1</sup>, Rodríguez, F.<sup>2</sup>, Destombe, C.<sup>3</sup> & Sourisseau, M.<sup>1</sup>

<sup>1</sup>Ifremer, Dyneco, Plouzané, France

<sup>2</sup>Instituto Español de Oceanografía (IEO, CSIC), Vigo, Spain

<sup>3</sup>*Station Biologique de Roscoff, CNRS, Sorbonne Université, Roscoff, France* mickael.le.gac@ifremer.fr

#### TUESDAY 22<sup>nd</sup> August 2023 Room « Tali ebrel »

Due to their expected extensive dispersal abilities in the marine environment, protist species are supposed to be cosmopolitan and to display homogeneous populations worldwide. However, strong genetic structure is extremely common in marine protist species. Understanding the evolutionary and ecological processes behind this structure is hindered by our ability to adequately sample the populations. Indeed, microbial census population sizes are often huge and difficult to apprehend. As a result, adequate sampling is a non-trivial problem. This problem is amplified by the need to isolate cells from natural samples and initiate clonal cultures to obtain sufficient genetic material for genotyping. Estimating allelic frequency directly from population wide samples, without any isolation step, offers an interesting alternative. Here, we validate the use of meta-transcriptome environmental samples to determine the population genetic structure of the dinoflagellate Alexandrium minutum. Strain and metatranscriptome based results both indicated a strong genetic structure for A. minutum in Western Europe. The presence of numerous private alleles, and even fixed polymorphism, would indicate ancient divergence and absence of gene flow between populations. Single nucleotide polymorphisms (SNPs) displaying strong allele frequency differences were distributed throughout the genome, which might indicate pervasive selection from standing genetic variation (soft selective sweeps). However, a few genomic regions displayed extremely low diversity that could result from the fixation of adaptive de novo mutations (hard selective sweeps) within the populations.

### Major contraction at warmer edges of the distribution of the coldwater algae *Himanthalia elongata* (Linnaeus) S.F. Gray 1821

Pereira, J.<sup>1,2,3</sup>, Monteiro, C<sup>1,3</sup>, Nieto Vilela, R<sup>4,5</sup> & Lima, F<sup>1,3</sup>

<sup>1</sup>CIBIO, Research Centre in Biodiversity and Genetic Resources - InBIO Associate Laboratory, Campus of Vairão, University of Porto, Vairão, Portugal <sup>2</sup>Laboratory, Country

<sup>2</sup>Department of Biology, Faculty of Sciences, University of Porto, Porto, Portugal

<sup>3</sup>BIOPOLIS Program in Genomics, Biodiversity and Land Planning, CIBIO, Campus of Vairão, Vairão, Portugal

<sup>4</sup>CIT Río Negro (CONICET), Sede Atlántica- Universidad de Río Negro, Rotonda Cooperación y Ruta Prov. N°1 (8500) Viedma Río Negro.

<sup>5</sup>Instituto de Biología de Organismos Marinos, LARBIM, CENPAT (CONICET), Bvd. Brown 2915 (U9120ACD), Puerto Madryn, Chubut - Argentina joanacp98@gmail.com

Global warming has been impacting marine ecosystems. Some of the most widespread consequences have been shifts in species distributions. A striking example is the case of the sea spaghetti, *Himanthalia elongata*, a canopy-forming brown macroalgae with an important structuring role in coastal ecosystems. This species is becoming extinct at its warm distribution edges – a phenomenon often associated with further biodiversity loss. Our objective was to reconstruct the historical changes in the distribution of *H. elongata* on the Atlantic coast of Europe – an area in which this species was repeatedly surveyed by renowned scientists and naturalists since the 1950s (E. Fischer-Piette, F. Ardré, etc.) – re-evaluating its current distribution and abundance and correlating distributional changes with remotely sensed and insitu temperatures collected by a network of autonomous loggers.

This algae used to occur from Scandinavia to central Portugal, with a distribution gap in the warmer Bay of Biscay. In the last decades, however, its distribution retreated hundreds of km northwards along the Portuguese coast, and currently, it can only be found in a single location. Additionally, Galician populations have declined, and the distributional gap in the Bay of Biscay has been widening eastwards. Here we will describe some drastic distributional shifts and explore the likelihood of their association with recent warming.

#### What is in the turf? A multi-approach biodiversity assessment of turfforming algae on rocky shores

Borer, G.<sup>1,2,3</sup>, Monteiro, C.<sup>1,3</sup>, Martins, F.M.S.<sup>1,3</sup> & Lima, F.P.<sup>1,3</sup>

<sup>1</sup>CIBIO, Research Centre in Biodiversity and Genetic Resources - InBIO Associate Laboratory, Campus of Vairão, University of Porto, Vairão, Portugal
 <sup>2</sup>Department of Biology, Faculty of Sciences, University of Porto, Porto, Portugal
 <sup>3</sup>BIOPOLIS Program in Genomics, Biodiversity and Land Planning, CIBIO, Campus of Vairão, Vairão, Portugal
 gabrielaborerrodrigues@gmail.com

Macroalgae provide vital functions in coastal ecosystems, with their composition and functionality varying significantly along latitudinal gradients. These communities are greatly affected by global warming, with macroalgal kelp forests now being replaced by simple and expanding "turf" communities, leading to unknown effects on associated biodiversity. To better understand the ecological role of these communities, the first step is to document the diversity of turf species present. However, current morphological methods present great challenges to their identification, leading to severe shortfalls in the geographical and taxonomical knowledge of certain taxa. This study applies morphological and molecular (metabarcoding) identification methods for assessing turf composition on rocky shores. Samples were collected at six study sites along the Portuguese coast, spanning three climatic regions. At each site, we performed in situ visual identifications of turfs in four biological replicates (quadrats), which were then carefully collected for molecular analysis. Since the lack of comprehensive DNA reference libraries limits the success of the molecular analysis, we also collected individual specimens visually identified for DNA barcoding. In this study, we aim to compare the efficiency of both methods in retrieving species diversity and composition of turfs. We also discuss in detail the advantages and challenges in developing a molecular method for studying such diversified communities, in the light of existing literature. This research intends to provide valuable insights into turf communities and uncover a better approach to assessing macroalgal community shifts in a global warming scenario.

#### In-depth characterization of diatom community succession over marked seasonal transitions in the subtropical Gulf of Aqaba-Eilat

Avrahami, Y.<sup>1,2</sup> & Frada, M.J.<sup>1,2</sup>

<sup>1</sup>The Interuniversity Institute for Marine Sciences in Eilat, Israel <sup>2</sup>Dept. of Ecology, Evolution and Behavior, Hebrew University of Jerusalem, Israel yoav.avrahami@mail.huji.ac.il

Diatoms are a main phytoplankton group, which are estimated to drive ~20% of global primary productivity. Diatoms often dominate high nutrient settings. However, they are found throughout the oceans including in oligotrophic areas. The Gulf of Aqaba/ Eilat (GoA) is a subtropical basin in the northeastern Red-Sea, where conditions markedly alternate between stratified and oligotrophic during summer and nutrient-richer during winter due to deep vertical-mixing. Cyanobacteria dominate the microbial community throughout summer. During winter, influx of nutrients enables increase in eukaryotes and the formation of a spring phytoplankton bloom, seemingly dominated by diatoms. Yet, the relative contribution of diatoms to autotrophic biomass, the diversity and seasonal succession remains poorly defined, limiting our understanding on the significance of such important primary producers in the GoA.

To address this knowledge gap, a routine bi-weekly sampling for phytoplankton and environmental parameters was undertaken in an offshore station in the Gulf during nearly 2 years. A dynamic and diverse assemblage of over 30 species was found. Major communities comprising different assortments of species and abundances characterized summer, mixing, and bloom communities. Generally, small diatoms ( $50\mu$ m >) dominated, with relevance for the pennate *Cylindrotheca closterium* and small centric Thalassiosiraceae. Chain-diatoms, particularly *Chaetoceros*, reached peak densities during the early mixing period and namely the spring bloom, encompassing ~50% of total diatoms, but rapidly dissipated at the onset of the stratified period. This study provides a baseline information for understanding the ecological role of diatoms in the Gulf's ecosystem and their adaptative responses to dramatic environmental fluctuations.

### Unveiling the hidden diversity of epiphytic diatoms associated with macroalgae *Padina* in the Indonesian Waters

Semba Prasetiya, F.<sup>1</sup>, Stewart, A.<sup>2</sup>, Ambar Prabowo, D.<sup>1</sup>, Radini Noerdjito, D.<sup>1</sup>, Purbani, D.C.<sup>1</sup>, Fahmi, V.<sup>1</sup>, Wiwin, N.<sup>3</sup>, & Mouget, J.L.<sup>2</sup>

<sup>1</sup>Research Center for Biosystematics and Evolution, Research Organization for Life Sciences and Environment, National Research and Innovation Agency (BRIN), Indonesia

<sup>2</sup>Laboratoire Biologie des Organismes Stress Santé Environnement (BiOSSE), Le Mans Université, France <sup>3</sup>Research Center of Biotechnology and Bioinformatics, Universitas Padjadjaran, Indonesia fidd001@brin.go.ig; fsembapr@gmail.com

Diatoms are important primary producers in aquatic ecosystems, and their biodiversity and species composition can provide valuable information about ecosystem functioning. This study aimed at investigating the diversity of epiphytic diatoms associated with the brown macroalgae *Padina*, in the Seribu Archipelago (Indonesia). A combination of classical morphological and DNA metabarcoding approaches was used to identify the diatom species associated with two distinct *Padina* species, *P. australis* and *P. minor*, from four sampling stations with varying nutrient loads.

The DNA metabarcoding approach allowed identifying four groups of diatoms, with the Bacillariophyceae family being dominant in all sampling stations. The study also revealed 57 diatom species common to all stations, with several endemic species found at each location. The Principal Component Analysis (PCA) showed that two distinct epiphytic diatom populations were associated with the two *Padina* species. Diatom species such as *Paralia longispina*, *Pseudictyota dubia*, *Halamphora* sp, *Nitzschia inconspicua*, *Flagilaria* sp, and *Microcostatus* sp were associated with *P. australis*, while *Chaetoceros* sp. and *Diploneis vacillans* were strongly associated with *P. minor*.

The study confirmed that DNA metabarcoding is a good alternative or a complementary tool to morphological identification for the study of phytoplankton communities. It also provided valuable information on the diversity and distribution of epiphytic diatoms associated with *Padina* species in the Seribu Archipelago, Indonesia. Furthermore, the results suggest that epiphytic diatoms have important ecological functions in the marine ecosystems, and their diversity and distribution could provide valuable information on their environmental quality.

### Marine heatwave susceptibility at the trailing edge of the foundational kelp, *Laminaria hyperborea*

Barreto, L.M.<sup>1</sup>, Serrão, E.A.<sup>1</sup>, Martins, N.<sup>1</sup>, Neiva, J.<sup>1</sup>, Bartsch, I.<sup>2</sup>, Valero, M.<sup>3</sup>, Franco, J.N.<sup>4</sup>, Jueterbock, A.<sup>5</sup>, Bárbara, I.<sup>6</sup>, de Clerck, O.<sup>7</sup> & Pearson, G.A.<sup>1</sup>

<sup>1</sup>Center of Marine Sciences (CCMAR) CIMAR, Portugal

<sup>2</sup>Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research, Germany

<sup>3</sup>Evolutionary Biology and Ecology of Algae, CNRS, Sorbonne Université, France

<sup>4</sup>MARE— Marine and Environmental Sciences Centre, ESTM, Portugal.

<sup>5</sup>Algal and Microbial Biotechnology Division, Faculty of Biosciences and Aquaculture, Nord University, Norway

<sup>6</sup>BioCost Research Group, Facultad de Ciencias and CICA, Universidade da Coruña, Spain <sup>7</sup>Phycology Research Group, Department of Biology, Ghent University, Belgium labarreto@ualg.pt

Suitable environmental conditions for foundation species such as kelp forests are currently being degraded by the increasing frequency, intensity and duration of marine heat waves (MHWs). It is therefore paramount to understand whether locally adapted populations can better withstand and survive these events across the species distributional range. There is a critical lack of studies focused on intraspecific variation in marine foundation species, potentially affecting the calculation of species vulnerability and compromising successful conservation and management strategies of entire ecosystems.

We studied *Laminaria hyperborea* as a model cold-temperate kelp species distributed across the Northeast Atlantic Ocean, which forms highly productive marine forests. We investigated population-level variation in response to a simulated MHW (interacting effects of temperature and nutrient availability), focusing on photosynthesis, growth, and survival of tissue from field-collected adult individuals among 3 populations representing 3 geographical regions within the distribution. Simulated marine heatwave events (19, 21 and 23°C) were performed in a temperature-controlled common garden experiment. Genetic diversity and differentiation among populations were assessed with microsatellite markers using a broader set of 12 populations.

We show that, despite being a highly diverse population harbouring unique genetic diversity, *L. hyperborea* from the warmer South edge suffered 100% post MHW mortality at the highest treatment temperature (23°C). North and Center populations were able to recover from 23°C after 18 days. Our results highlight the need to carefully assess intraspecific variation, and the potential for local adaptation, within a biogeographical framework.

### Integrating metabolomics and DNA to uncover patterns of diversity in two sister species of kelp

Reddy, M.M.<sup>1,2</sup>, Greff, S.<sup>3</sup>, McCann, M.<sup>2</sup>, Bolton, J.J.<sup>3</sup> & Thomas, O.P.<sup>2</sup>

<sup>1</sup>Department of Biological Sciences, University of Cape Town, South Africa

<sup>2</sup>Marine Biodiscovery, School of Biological and Chemical Sciences and Ryan Institute, National University of Ireland Galway, Ireland

<sup>3</sup>Institut Méditerranéen de Biodiversité et d'Ecologie Marine et Continentale (IMBE), UMR 7263 CNRS, IRD, Aix Marseille Université, Avignon Université, Station Marine d'Endoume, rue de la Batterie des Lions, Marseille, France

maggiereddy0402@gmail.com

#### TUESDAY 22<sup>nd</sup> August 2023 Room « Tali ebrel »

Kelp forests are among the most productive ecosystems in our oceans, supporting biodiverse and commercially important species. The only major kelp forest in Sub-Saharan Africa occurs in Southern Africa, where these habitats are formed largely by species of Laminaria and Ecklonia. Two species of *Ecklonia* are widespread, with *E. maxima* dominant along cool temperate coastlines to the west, and *E.* radiata occurring along warm temperate regions to the east, including nearby offshore reefs. More recently, an "Ecklonia contact zone" has formed at the southern tip of Africa, following a recent eastward shift in the range of *E. maxima*. The aim of the present study was to elucidate the chemical and genetic diversity of E. maxima and E. radiata in southern Africa and to determine whether these two species hybridize. This was achieved by using a combined metabolomic and DNA approach applied to populations of Ecklonia collected from throughout its known distribution range in Southern Africa spanning roughly 3500 km of coastline. We used untargeted metabolomics by LCMS/MS and cox1, with an additional gene applied to selected samples, to estimate their overall chemical and genetic diversity respectively. Our data largely resolved distinct biogeographic groups within each species and supported a third distinct group intermediate between E. maxima and E. radiata. Our combined metabolomic and DNA approach was powerful in elucidating patterns of diversity and structure in Ecklonia along an environmentally dynamic coastline, and we believe that such information can help guide MPA planning and management in the future.

### Interannual variability of genetic and demographic structure in blooms of the marine diatom *Pseudo-nitzschia multistriata*

Ruggiero, M.V.<sup>1</sup>, Buffoli, M.<sup>1</sup>, Wolf, K.<sup>2</sup>, D'Alelio, D.<sup>1</sup>, Lombardi E.<sup>1</sup>, Manfellotto, F.<sup>1</sup>, Margiotta, F.<sup>3</sup>, Sarno, D.<sup>3</sup>, Vitale, L.<sup>1</sup>, John, U.<sup>4</sup>, Ferrante, M.I.<sup>1,5</sup> & Montresor, M.<sup>1</sup>

<sup>1</sup>Department of Integrative Marine Ecology, Stazione Zoologica Anton Dohrn, Italy

<sup>2</sup>Institute of Marine Ecosystem and Fishery Science Plankton Ecology and Evolution; Universität Hamburg, Germany

<sup>3</sup>Department of Research Infrastructures for Marine Biological Resources, Stazione Zoologica Anton Dohrn, Italy

<sup>4</sup>Biosciences | Ecological Chemistry, Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research, Germany

<sup>5</sup>Oceanography Section, National Institute of Oceanography and Applied Geophysics (OGS), Trieste, Italy marina.montresor@szn.it

Studies carried out at different spatial scales demonstrated the presence of genetic structure in planktonic unicellular eukaryotes, but information on the genetic structure of populations at the temporal scale is still limited. We present the results of a multiannual genetic characterization of blooming populations of the marine planktonic diatom *Pseudo-nitzschia multistriata* in the Gulf of Naples. Fingerprinting of more than 1000 isolated strains was carried out with microsatellites and was flanked in some years by Microsatellite Pool-seq Barcoding, which is based on Illumina sequencing of microsatellite loci in natural samples. The two approaches provided comparable results, indicating a clonal expansion event in late summer-autumn 2013 and a more stable genetic structure in the period 2017-2020 than in the previous years.

*Pseudo-nitzschia multistriata* is a heterothallic diatom for which the mechanism of mating type (MT) determination has been recently elucidated. This allowed MT assignment by PCR in 980 strains collected over the years. MTs were generally at equilibrium with two major exceptions, one in 2008 and the other during the clonal bloom of 2013, in which MT+ was found to be predominant. Index of

association ( $I^A_s$ ) showed that that the population was in linkage equilibrium in most of the blooms, suggesting that sexual reproduction could balance the clonal expansion occurring in some years. Our results demonstrate that the genetic and demographic structure within *P. multistriata* blooms can be markedly different among years raising several questions that can be addressed with further genomic approaches.

### Automated Environmental Sampler and Laboratory-on-a-Chip to Enhance Monitoring for Toxic Algae

Medlin, L.K.<sup>1</sup> & Faith, M.<sup>2</sup>

<sup>1</sup>Algae Save the World, Consulting, UK <sup>2</sup>University of Plymouth, UK

Novel sampling and organismal detection systems have been developed to take a larger, more representative sample whose concentrate can be used for DNA/RNA extractions for species detection for better monitoring results to facilitate policy decision. An automated environmental sampler that uses kidney dialysis hollow fibre filters to concentrate all organisms from 25-50 litres into a 1 litre concentrate for downstream DNA/RNA extraction and analysis. The taking of such a large volume of water for any environmental sample provides a much broader picture of the ecosystem to be sampled/monitored. This broader picture will pick up more information on rare events and allow policy decisions to be made based on samples that are more representative of the natural environment than samples of 1 L or less that are now routinely taken by environmental scientists and monitoring agencies. From the concentrated environmental sample, RNA is extracted and used in biosensors with electrochemical detection for toxic algae. Single electrode chips are being developed into a 16-electrode laboratory-on-achip. Our long-term goal is to develop an early warning system for toxic algae in a miniaturised laboratory on a chip format with multiplexed electrodes with barcodes for 160 toxic algal species. The barcodes in a single electrode format show a high signal and no cross reactivity when challenged with non-target RNA. Plans are to combine the environmental sampler and the laboratory on a chip into one instrument that will afford real-time monitoring of the environment. We seek collaborations to develop the laboratory on a chip with 144 multiplexed electrodes.

#### SYMPOSIUM 06 "Algal cell biology and ultrastructure"

#### **KEYNOTE**

#### Cell walls and their remodelling in green algae

#### Herburger, K.

Institute of Biological Sciences, University of Rostock, Germany klaus.herburger@uni-rostock.de

The cells of most green algae are surrounded by a primary cell wall, which is an extendable matrix constructed from various polysaccharides and glycoproteins, and can extend into mucilaginous sheaths. Cell walls are the bulk material in green biomass and are crucial for algae's function and survival because they enable multicellularity by adhering cells together, direct cell expansion, and act as the only physical barrier between the sensitive algal protoplast and the environment. There is increasing evidence that these key cell wall functions are supported by wall-resident enzymes, which, for example, can recruit new polysaccharides into the wall and reconnect them with others nearby. Such enzyme activities underwent a strong expansion in land-conquering charophytes (Zygnematophyceae) and became a fundamental part of the land plant cell wall metabolism. This talk will discuss the potential "original" functions of cell wall remodelling in green algae and highlight differences from their descendants, the land plants. Moreover, I will discuss novel biorthogonal imaging techniques that allow tracking of specific cell wall components and studying cell wall remodelling and formation *in vivo*.

#### ORALS

#### Characterisation of alginate lyases from brown algae

Bouguerba-Collin, A.<sup>1</sup>, Mazeas, L.<sup>1</sup>, Cock, M.<sup>1</sup>, Terrapon, N.<sup>2</sup> & Hervé, C.<sup>1</sup>

<sup>1</sup>Sorbonne Université Paris 06, CNRS, UMR 8227, Integrative Biology of Marine Models, Station Biologique de Roscoff, Roscoff, France. <sup>2</sup>CNRS, Aix Marseille Univ, UMR 7257 AFMB, 13288 Marseille, France ahlem.bouguerba@sb-roscoff.fr

The extracellular matrix (ECM) in brown algae is involved in many functions including cell development, communication between cells and defense responses. It features a unique biochemistry among eukaryotes, with the gel-forming alginates being a main component, and often seen as an analogue to pectins in plants. The first brown algal genome was obtained in 2010 and allowed predictions on cell-wall biogenesis. Possible enzymes involve in the ECM expansion were unknown, until the recent biochemical characterization of an alginate lyase from a brown alga, that shaped a novel PL41 family. As a partner in the Phaeoexplorer consortium, we have now annotated the carbohydrate active enzymes (CAZymes) of 40 brown algal genomes. Our analyses indicate that the PL41s shape a multigenic family in brown algae with a phylogeny made of distinct clades and transcriptomic variations upon lifecycle. Most of these enzymes are multimodular, with putative alginate-binding domains. These results suggest distinct enzyme specificities that may be related to ECM processing. In order to validate functionally those genes, I am assaying the recombinant expression and biochemical characterization of

all PL41s from *Ectocarpus* sp.7. Altogether, these results will allow to expand our understanding of alginate remodelling in brown algae.

### Synchrotron X-rays reveal new insight into halogen and trace metal storage in brown algae

Küpper, F.C.<sup>1,2,3</sup>, Mijovilovich, A.<sup>4</sup>, Cloetens, P.<sup>5</sup>, Kloareg, B.<sup>6</sup>, Kroneck, P.M.H.<sup>7</sup>, Newville, M.<sup>8</sup>, Lanzirotti, T.<sup>8</sup>, Miller, E.P.<sup>3</sup>, Kumari, P.<sup>1</sup>, Feiters, M.C.<sup>9</sup>, Meyer-Klaucke, W.<sup>10</sup>, Katsaros, C.<sup>11</sup>, Küpper, H.<sup>4,12</sup> & Carrano, C.J.<sup>3†</sup>

<sup>1</sup>School of Biological Sciences, University of Aberdeen, Cruickshank Building, St. Machar Drive, Aberdeen AB24 3UU, Scotland, UK.

<sup>2</sup>Marine Biodiscovery Centre, Department of Chemistry, University of Aberdeen, Aberdeen AB24 3UE, Scotland, UK.

<sup>3</sup>Department of Chemistry and Biochemistry, San Diego State University, CA, 92182-1030, USA.

<sup>4</sup>Czech Academy of Sciences, Biology Centre, Institute of Plant Molecular Biology, Laboratory of Plant Biophysics and Biochemistry, Branišovská 31/1160, 370 05 České Budějovice, Czech Republic.

<sup>5</sup>ESRF – The European Synchrotron, beamline ID16A, Grenoble, France.

<sup>6</sup>Sorbonne Université, CNRS, Integrative Biology of Marine Models (LBI2M), Station Biologique de Roscoff (SBR), 29680 Roscoff, France.

<sup>7</sup>Department of Biology, University of Konstanz, Universitätsstrasse 10, 78457, Konstanz, Germany.

<sup>8</sup>Center for Advanced Radiation Sources, The University of Chicago, Chicago, IL, USA.

<sup>9</sup>Dept. of Organic Chemistry, Institute for Molecules and Materials, Radboud University Nijmegen, Heyendaalseweg 135, NL-6525 AJ Nijmegen, The Netherlands.

<sup>10</sup>Deutsches Elektronen Synchrotron DESY, Hamburg, Germany.

<sup>11</sup>Department of Biology, National and Kapodistrian University of Athens, Panepistimiopolis, Athens 157 84, Greece.

<sup>12</sup>University of South Bohemia, Department of Experimental Plant Biology, Branišovská 31/1160, 370 05 České Budějovice, Czech Republic.

fkuepper@abdn.ac.uk

<sup>†</sup>We dedicate this paper to the memory of Prof. Carl J. Carrano (14 July 1950 – 26 January 2022). He will be remembered for his many inspiring contributions to inorganic biochemistry especially of marine organisms.

The combination of nanoprobe XRF tomography with  $\mu$ XANES, allowing detection of localisation in combination with chemical speciation at subcellular resolution, is a potent tool to reveal new insight about the mechanistics of metal and halogen storage in algae. The presentation will include several case studies of iodine, bromine, iron, strontium, zinc and arsenic in *Laminaria digitata* and *Ectocarpus siliculosus*.

Using  $\mu$ XRF nanoprobe tomography, we unambiguously show that iodide in *Laminaria* is stored in intracellular vesicles of cortical cells. In contrast, bromine is mostly accumulated in the vacuoles of meristoderm cells. Furthermore, using  $\mu$ XANES, this study provides first evidence for the formation of either molecular I<sub>2</sub> or an organo-iodine compound as a minor component in the cortical cell layer.

Fe distribution shows higher accumulation in the cortex and symplastic subcellular localisation. This agrees with recent ultrastructural insight by cryofixation–freeze substitution that found a new type of cisternae which may have a storage function but differs from the apoplastic Fe accumulation found by conventional chemical fixation. Zn distribution colocalizes with Fe in *E. siliculosus* whereas it is chiefly located in the *L. digitata* medulla, which is similar to As and Sr. Both As and Sr are found mostly at the cell wall of both algae. XANES spectra indicate that Fe in *L. digitata* is stored in a mineral non-ferritin

#### TUESDAY 22<sup>nd</sup> August 2023 Room « Tali moan »

core, due to the lack of ferritin encoding genes. We hypothesize that the *L. digitata* cortex contains mostly a ferritin-like mineral while the meristoderm contains mostly Fe-S.

#### ROS signaling in red algae

#### Kim, G.H.

Department of Biology, Kongju National University, Korea ghkim@kongju.ac.kr

Initially considered as toxic byproducts of aerobic metabolism, Reactive Oxygen Species (ROS) have emerged as major regulatory molecules in organisms. We found that the elaborate wound regeneration and unique fertilization process of red algae, which involves the interaction of multiple complementary cells, provides an interesting model to study ROSmediated signaling. We visualized ROS generation in repair cells using DCFH-DA staining, identified the genes involved in wound regeneration and fertilization in two red algal species, *Griffithsia monilis* and *Bostrychia moritziana*, and examined their expression using quantitative PCR. During fertilization, ROS were instantaneously produced by spermatia (sperm) when they attached to female trichogynes (egg receptive area), diffused across the cell membrane in the form of H<sub>2</sub>O<sub>2</sub>, and triggered ROS generation in the carpogonium (egg) as well as carpogonial branch cells which are not in direct contact with spermatia. During wound healing, ROS act as plant hormones that induce the division of repair cells and attract the tips of the repair cells to extend towards each other, ultimately leading to somatic fusion.

Repair cells elongate along the  $H_2O_2$  gradient, meet at each other's tips where the  $H_2O_2$  concentration is highest, and undergo somatic fusion. Our results suggest that  $H_2O_2$  acts as an auto-propagating signaling molecule, possibly through  $Ca^{+2}$  channel activation, and regulates gene expression involved in fertilization as well as wound healing in red algae.

#### Mapping of subcellular local pHs in marine diatoms

Shimakawa, G., Yashiro, E. & Matsuda, Y.

School of Biological and Environmental Sciences, Kwansei Gakuin University, Japan gshimakawa@kwansei.ac.jp

Diatom (Bacillariophyceae), the algal group categorized to heterokonts, is one of the most important secondary algae on the earth that comprises tens of thousands of species and that contribute to about 20% of the global primary production. Because of serial endosymbiotic events and horizontal gene transfer, diatoms develop the "secondary plastid" bounded by four membranes and unique metabolic features. Further, the secondary plastid contains the phaseseparated microcompartment, termed as the pyrenoid, which comprises of densely packed ribulose 1,5-bisphosphate carboxylase/oxygenase. Thylakoid membranes harboring photosynthetic electron transport components are located in peripheral regions of the chloroplast surrounding the pyrenoid, and a part of which is penetrated into the pyrenoid. However, the physiological significances of the unique structure of diatom chloroplasts have never been understood. Characterization of fundamental physiological parameters such as local pHs at the various subcellular compartments should help the understandings of the physiological roles of the unique structure of the secondary plastid. A promising method to estimate local pHs is the *in situ* expression of the pH-sensitive green fluorescent protein. Here, we first developed the molecular tool for the mapping of *in situ* local pHs in the diatom *Phaeodactylum tricornutum* by heterologously expressing pHluorin2 in the cytosol, periplastidal compartment (the space in between each two outer and inner chloroplast

envelopes), chloroplast stroma, and the pyrenoid matrix. Our data suggested that periplastidal compartment and the pyrenoid matrix are more acidic than their adjacent areas, the cytosol and the chloroplast stroma.

### 3D reconstruction of *Spirogyra* and *Zygnema* (Charophyta) zygospores reveals new details on maturation and cell wall formation

Holzinger, A.<sup>1</sup>, Permann, C.<sup>1</sup>, Antreich, S.J.<sup>1</sup>, Herburger, K.<sup>2</sup>, Jouneau, P-H.<sup>3</sup>, Uwizeye, C.<sup>3</sup>, Falconet, D.<sup>3</sup> & Maréchal, E.<sup>3</sup>

<sup>1</sup>Department of Botany, University of Innsbruck, Austria

<sup>2</sup>Institute of Biological Sciences, University of Rostock, Germany,

<sup>3</sup>Interdisciplinary Research Institute of Grenoble (IRIG), University Grenoble Alps, CEA, CNRS, Grenoble, France

Andreas.Holzinger@uibk.ac.at

Strategies of aquatic green algae to conquer land can be found in Zygnematophyceae, the sister clade of land plants. These green algae reproduce sexually by conjugation leading to the formation of resistant zygospores. They undergo a maturation process, where the male chloroplast is terminated. In both investigated species (Spirogyra sp. and Zygnema vaginatum), the zygospores are formed inside the female gametangia, but differ in the thickness of the mesospore. Serial block face-scanning electron microscopy (SBF-SEM) was applied on high pressure frozen/freeze substituted embedded samples of different Spirogyra sp., focussed ion beam SEM (FIB-SEM) on chemically fixed Zygnema vaginatum zygospores. Both techniques allowed a reconstruction of the zygospore formation at different maturation stages. In Spirogyra sp., chloroplasts were arranged as helices, and the male chloroplast was aborted and probably converted into vacuole-like compartments pre-vacuoles with a medium electron density. During zygospore ripening, Spirogyra sp. zygospores strongly upregulate lipid production and energy storage for later germination. In Zygnema, less lipid droplets are found and a massive, layered mesospore is formed. The visualization of 3D chloroplast architecture, the pyrenoid with inner thylakoids in gyroid arrangement, and the distribution of the large mitochondria, provide new insights in the dynamic reorganization processes during zygospore maturation. The unique cell wall architecture of both investigated genera is a structural innovation that permitted a shift to terrestrial habitats marked by frequent episodes of dryness. The acquisition of such structural feature represents a major evolutionary step towards land conquest by plants.

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### Detailed observation of secondary chloroplast division in *Phaeodactylum tricornutum*

#### Tanaka, A.

Algal Functional Morphology Laboratory, Faculty of Science, University of the Ryukyus, Japan \*tanakaa@sci.u-ryukyu.ac.jp

Secondary chloroplast established by two-times endosymbiosis plays a significant role in carbon fixation in aquatic environments. In contrast to the primary chloroplasts of land plants and red algae,

#### TUESDAY 22<sup>nd</sup> August 2023 Room « Tali moan »

the dividing mechanism of the secondary chloroplasts has not been completely clarified yet due to their quadruple membranes. Indeed, no clues are known about the dividing machinery for the outer two membranes, and the availability of the dividing machinery inherited from the primary chloroplast is still controversial. Therefore, as a first step to understanding the secondary chloroplast division, the behavior of the outer two membranes during chloroplast division and the evidence of involvement of a dynamin-related protein 5B (DRP5B) will be shown by using a diatom *Phaeodactylum tricornutum* in this presentation.

Observation with transmission electron microscopy elucidated that the chloroplast in *P. tricornutum* was bisected in the mid-part, where a central pyrenoid locates. More than one ER (not CER) was located parallel to the dividing plane in the contracting site, and double-aligned particles were observed inside the ERs. In another cell, a chloroplast including a pyrenoid had already divided by inner two membranes, but the outer CER stayed on the surface. Interestingly, the novel membranous separation between two daughter chloroplasts was seemly formed centrifugally by vesicle supply from the CER. In addition, the involvement of DRP5B in the chloroplast division was demonstrated by the dysfunction of chloroplast division in the DRP5B dominant negative strain.

#### **KEYNOTE**

#### Using cryo electron microscopy to study diatom silicification

#### Gal, A.

Dept. of Plant and environmental Sciences, Weizmann Institute of Sciences, Israel assaf.gal@weizmann.ac.il

The process of diatom silicification, which gives rise to their exquisite silicified cell walls, is under strict biological control. This is an inorganic polymerization process that occurs intracellularly within highly confined environments. Our understanding of the structural and chemical aspects of diatom silicification is still rudimentary, as conventional imaging and analytical tools are inadequate to resolve the native-state conditions related to mineral formation processes. In our work, we use a suite of cryo electron microscopy techniques to extract native-state information with nanoscale resolution. Our direct approach to study the formation of diatom silica *in situ* yields detailed understanding of the cellular controls over the silicification process in diatoms. In this presentation, I will elaborate on the functional roles of membrane interactions in controlling silica formation, from morphogenesis to exocytosis of the silica elements, and on how imaging approaches can open a window into the cell biology of these extraordinary microorganisms.

#### ORALS

### Cell wall evolution in diatoms: how are shape, size and silicification involved in frustule adaptive functions?

Petrucciani, A.<sup>1</sup>, Chaerle, P.<sup>2</sup>, Knoll, A.H.<sup>3</sup> & Norici, A.<sup>1</sup>

<sup>1</sup>Dipartimento di Scienze della Vita e dell'ambiente, Università Politecnica delle Marche, Italy <sup>2</sup>BCCM/DCG Diatoms Collection, Department of Biology, Ghent University, Ghent, Belgium <sup>3</sup>Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA, United States a.petrucciani@staff.univpm.it In present oceans, diatoms represent the most abundant and diversified class of primary producers distinguished by the ability to incorporate silicic acid in a silica outer shell called frustule. By the Cretaceous Period, large spherical diatoms with highly silicified frustules thrived in surface oceans; only later, while occurring a drastic Si decline, species with elongated and thinner frustules (lower  $SiO_2$  content) radiated. Numerous adaptive functions are ascribed to frustules, including protection against predators, enhancement of nutrient uptake, control of vertical movements through the water column.

Given the vast diversity of frustules in terms of shape, size and silicification, how does evolved traits rule the adaptive functions? To answer the question, diatoms were acclimated to reconstructed paleoenvironments mimicking Si decline to assess it as a selective pressure on species radiation. Moreover, the interaction of morphologically distinct diatoms with competitors and predators was investigated; their *in vivo* sinking capacity was also studied.

The observed physiological responses of pennates to different Si regimes favoured their differential diversification, governing reduced size and Si usage in diatoms as a group. Regarding adaptive functions, elongated shape benefits cells in escaping from predators also when facing competition for resources. On the other hand, frustule silicification controls species vertical movements more than other morphological characteristics. Nevertheless, a higher energy (light) availability enhances cell floating independently from silica content, indicating buoyancy control is strongly tuned by cell metabolism.

Through physiological experiments, the entire study points out that evolution has shaped competitive frustule traits distinctly involved in adaptive functions.

### Morphological and physiological remodelling of *Chlamydomonas reinhardtii* zygotes during zygospore formation

Cardador, M.<sup>1</sup>, Dunker, S.<sup>2,3</sup>, Krüger, S.<sup>3</sup>, Jakob, T.<sup>1</sup>, Goss, R.<sup>1</sup> & Sasso, S.<sup>1,3</sup>

<sup>1</sup>Department of Plant Physiology, University of Leipzig, Germany

<sup>2</sup>Helmholtz Centre for Environmental Research (UFZ), Leipzig, Germany

<sup>3</sup>German Centre for Integrative Research (iDiv), Leipzig, Germany

<sup>4</sup>Biozentrum, Microscopy Unit, Martin Luther University of Halle-Wittenberg, Halle (Saale), Germany martim.cardador@uni-leipzig.de

The process of maturation, which transforms Chlamydomonas reinhardtii zygotes into dormant zygospores, has thus far been poorly explored. In the present study, the morphological and physiological changes of the zygospore during maturation were analysed. Imaging flow cytometry showed that the cell diameter of the zygote population increased, resulting in a 2-fold increase in cell volume after 28 days of maturation. The increase in cell volume was accompanied by a change in cell shape and it was observed that the cells became progressively more spherical up to 7 days. Analysis of the macromolecular composition of the zygote biomass demonstrated that the change in cell volume coincided with 2- and 3-fold increases of the lipid and carbohydrate fractions of the cells, respectively, while the protein content decreased 2-fold. An analysis of the cell lipids demonstrated that the higher lipid content was caused by the accumulation of triacylglycerol. Regarding the physiology of the zygospores, it was found that photosynthetic activity declined in conjunction with the degradation of photosynthetic pigments. Interestingly, chlorophyll degradation was not uniform throughout the zygote population, as demonstrated by the residual chlorophyll fluorescence in part of the zygote cells. Electron microscopy also revealed that the chloroplast remained present in the zygospore cell even after 28 days. In conclusion, the present study demonstrates that the C. reinhardtii zygote undergoes a global morphological and physiological changes as it becomes a dormant and resistant zygospore.

#### Light Focusing in Pennate Diatoms: Can Living Diatoms See?

Ghobara, M.<sup>1</sup>, Oschatz, C.<sup>2</sup>, Gilic, M.<sup>1</sup>, Fumagalli, P.<sup>1</sup> & Reissig, L.<sup>1</sup>

<sup>1</sup>Institute of Experimental Physics, Freie University Berlin, Arnimallee 14, 14195 Berlin, Germany. <sup>2</sup>Max Planck Institute of Colloids and Interfaces, Department of Biomaterials, Research Campus Golm, Am Mühlenberg 1, 14476 Potsdam, Germany. mohamedghobara@rockemtail.com

The light-focusing ability of valves and frustules of a few centric and pennate species has been numerically and experimentally investigated and attributed to diffraction and interference, where valves act as microlenses. The correlation between the reported focusing behavior and photobiology is unclear yet. Most assume it enhances the photosynthetic process. In this work, we employed numerical analysis and experimental techniques, including scanning near-field optical microscopy (SNOM) and normal-and sideillumination optical microscopy, to elucidate the focusing abilities of the thicker parts within biraphid pennate valves, such as nodule zone, sternum, and helictoglossa. Our numerical results suggest the ability of these structures to focus light in the near-field inside the living cells, with higher intensity at higher frequencies (e.g. blue light). Moreover, the direction of the focusing beam follows the incident light direction. The experimental results confirm such focusing behavior. The results obtained suggest that this type of light focusing is probably not primarily relevant to a putative vision mechanism, similar to the suggestions that have been made for unicellular cyanobacterium *Synechocystis* sp., and may help to explain the signal transduction cascade of the phototactic mechanism in biraphid pennates.

### Understanding calcium signalling pathways in diatom chloroplasts in response to high light and oxidative stress

Wheeler, G.<sup>1</sup>, Flori, S.<sup>1</sup>, Dickenson, J.<sup>1</sup>, Kleiner, F.<sup>1</sup>, Helliwell, K.<sup>1,2</sup>, Brownlee, C.<sup>1</sup>

<sup>1</sup>The Marine Biological Association, Plymouth, UK <sup>2</sup>The University of Exeter, Exeter, UK glw@mba.ac.uk

Diatoms are a group of silicified phytoplankton that play an important role in many marine and freshwater ecosystems. Like many eukaryotes, diatoms utilise calcium-dependent signalling mechanisms in order to respond to changes in their environment. We have recently examined how diatoms use cytosolic calcium signals in response to many stimuli, including salinity, temperature and nutrient availability. However, the nature of calcium-dependent signalling pathways in other cellular compartments in diatoms remain largely unknown. We have examined chloroplast calcium signalling in the diatom *Phaeodactylum tricornutum*. We find that chloroplast calcium is not elevated by stimuli that induce large cytosolic calcium elevations, such as hypo-osmotic stress. In contrast, we find that high light and exogenous hydrogen peroxide induce large sustained calcium elevations in the chloroplast stroma that are not replicated in the cytosol. Measurements of hydrogen peroxide indicate that chloroplast calcium elevations induced by these stimuli correspond to the accumulation of hydrogen peroxide in the chloroplast. Chloroplast calcium elevations were also induced by the addition of other oxidants, including methyl viologen, which acts to generate superoxide within the chloroplast. Our findings demonstrate that the diatom chloroplast acts as an independent calcium signalling compartment

and suggest that calcium-dependent signalling pathways within this organelle play an important role in the response of diatoms to photo-oxidative stress.

#### Mixed messages: The influence of nitrate and phosphate limitation on nutrient sensing in model marine diatoms.

Harrison, E.L.<sup>1,2</sup>, Meeda, Y.<sup>1,2</sup>, Gaikwad, T.<sup>1</sup>, Wheeler, G.<sup>1</sup> & Helliwell, K.E.<sup>1,2</sup>

<sup>1</sup>Marine Biological Association, Citadel Hill Plymouth, PL1 2PB <sup>2</sup>University of Exeter, Exeter EX4, 4QD ellhar@mba.ac.uk

Diatoms are an integral part of the marine ecosystem, accounting for 20% of global primary production. In the marine environment they can encounter highly variable nutrient regimes across a range of spatiotemporal scales. Nitrogen (N) and Phosphorus (P) are two key nutrients, necessary for diatom growth, that through changing human activities are of concern in both the marine environments with agricultural run-off leading to algal blooms damaging aquatic ecosystems and on land in terms of adequate nutrient supply for crop growth. It is therefore crucial to understand more about how microalgae, including diatoms, sense and respond to various nutrient limitation or resupply scenarios. The use of CRISPR-Cas9 and genetically encoded  $Ca^{2+}$  biosensors has enabled the demonstration of rapid  $Ca^{2+}$  elevations in response to P resupply in diatoms starved of P. When this pathway is activated there is also upregulation of N uptake and assimilation machinery, suggesting crosstalk, as found in plants, to balance N and P status within the diatom cells. We aim to investigate further how colimitation of N and P alters nutrient physiology and signalling of two model marine diatoms, *Phaeodactylum tricornutum* and *Thalassiosira pseudonana*. In particular, how N and P co-limitation affects  $Ca^{2+}$  signaling in response to nutrient resupply will be explored, in an effort to further characterize how marine diatoms are able to rapidly respond to changing nutrient pulses in their natural environment.

# Nitrogen fixation in the form of guanine crystals and rearrangements of crystals as an adaptation for various illumination levels of *Amphidinium carterae*

Bokov, M.<sup>1</sup>, Hodoš, M.<sup>1</sup>, Pilátová, J.<sup>1,2</sup> & Mojzeš, P.<sup>1</sup>

<sup>1</sup>Institute of Physics, Faculty of Mathematics and Physics, Charles University, Ke Karlovu 5, CZ-121 16 Prague 2, Czech Republic

<sup>2</sup>Department of Experimental Plant Biology, Faculty of Science, Charles University, Prague, Czech Republic maxibokov2@gmail.com

The utilization of biogenic guanine crystals by animals to manipulate light is a wellknown phenomenon. Guanine crystal arrays possess a high refraction index and plate-like arrangement, enabling them to function as diffuse scatterers, reflectors, tunable photonic crystals, and image-forming mirrors. Recently, crystalline inclusions comprising guanine or related purines have been identified in various phylogenetically unrelated photosynthetic microalgae. In *Amphidinium carterae*, a dinoflagellate, guanine crystals were observed to serve as a longterm, high-capacity store of nitrogen, although their role in light manipulation had been previously speculated for photosynthetic eukaryotes. Confocal Raman microscopy was employed to visualize crystalline guanine within intact *A. carterae* cells, revealing that the location of guanine crystals was dependent on illumination intensity. Regardless of the light conditions, the initial position of crystal formation is inside and at the periphery of the cell

TUESDAY 22<sup>nd</sup> August 2023 Room « Tali moan »

membrane. However, under low light intensity (ca 3-5  $\mu$ mol(photons) m<sup>-2</sup> s<sup>-1</sup>), guanine crystals were synthesized closer to the center of the cell, acting as photonic mirrors or diffusers to enhance the efficiency of photosynthesis. Conversely, high light intensity (up to 500  $\mu$ mol(photons) m<sup>-2</sup> s<sup>-1</sup>), led to the organization of extensive guanine crystal layers between the cell wall and plastids, providing protection against excessive illumination and regulating photosynthetic efficiency. This study highlights the potential role of guanine crystals in regulating photosynthetic processes and protecting plastids from photo damage in *A. carterae*.

### Diatoms nitrate transporters as regulators of intracellular nitrogen dynamics in a changing environment

Santin, A.<sup>1,2</sup>, Croteau, D.<sup>3</sup>, Chiurazzi, M.<sup>4</sup>, Ribera d'Alcalá, M.<sup>1</sup>, Bailleul, B.<sup>3</sup>, Falciatore, A.<sup>3</sup>, Russo, M.T.<sup>1</sup>, Ferrante, M.I.<sup>1,5</sup> & Rogato, A.<sup>1,4</sup>

<sup>1</sup>Stazione Zoologica Anton Dohrn, Naples, Italy
<sup>2</sup>University of Padua, 35121 Padua, Italy
<sup>3</sup>Institut de Biologie Physico-Chimique, UMR7141, CNRS-Sorbonne Université, Paris, France
<sup>4</sup>Institute of Biosciences and BioResources, CNR, Naples, Italy
<sup>5</sup>National Institute of Oceanography and Applied Geophysics, 34010 Trieste, Italy
alessandra.rogato@ibbr.cnr.it

Diatoms are among the most successful microalgae in facing environmental changing. They own unique features that confer them the capability to sense both extracellular and intracellular conditions and quickly respond to them. Among these strategies, the intracellular re-allocation of resources, that could be stored, moved and used according to cellular needs, is mediated by transporters, which are still poorly known in diatoms.

An interesting class of transporters is represented by the Nitrate Transporter 1 / Peptide Transporter Family (NPFs), widely studied in many organisms, where they can recognize a broad range of substrates, ranging from di- and tri-peptides in bacteria to a wide variety of molecules including nitrate in higher plants.

To get insights into how the evolutionary divergence of diatom NPFs in two distinct groups could result in a functional diversification, we took advantage of the model species *Phaeodactylum tricornutum*, which owns two NPFs, called PtNPF1 and PtNPF2, and we generated gain and loss of function mutants. Characterization of PtNPF1 suggests that it is localized on the vacuole membrane, acting as sensor and transporter of vacuole-stored nitrate for intracellular nitrogen re-allocation. On the other hand, data on PtNPF2 indicate that it could be involved in proton transport between chloroplast membranes. Indeed, growth phenotype observed in knock-out mutants at low pH, suggests that PtNPF2 could play a role in intra-cellular pH homeostasis.

Knowing the mechanisms used by diatoms to regulate their intracellular metabolism represents an important contribution for understanding their ecological and evolutionary success and evaluate their potential to adapt to environmental changes.

### The phase separated CO<sub>2</sub>-fixing *Chlamydomonas* pyrenoid proteome determined by TurboID

Lau, J.S., Dowle, A., Gavin, T.H., Girr, P. & Mackinder, L.C.M.

Department of Biology, University of York, United Kingdom justin.lau@york.ac.uk

Phase separation underpins many biologically important processes such as RNA metabolism, signaling and CO<sub>2</sub> fixation. However, the composition of a phase separated organelle is often challenging to determine as their sensitivity to environmental conditions limits the application of traditional proteomics techniques like organellar purification or affinity purification mass spectrometry. In Chlamydomonas reinhardtii, Rubisco is packaged into a crucial phase separated organelle called the pyrenoid that improves photosynthetic performance by supplying Rubisco with elevated concentrations of CO<sub>2</sub>. This organelle is found to occur in all algal lineages, further suggesting their biogeochemical importance. We developed a TurboID based proximity labeling technique in the Chlamydomonas chloroplasts, where proximal proteins are labeled by biotin radicals generated from the TurboID-tagged protein. By expressing two core pyrenoid components fused with the TurboID tag, we have generated a high confidence pyrenoid proxiome that contains the majority of known pyrenoid proteins and several novel pyrenoid candidates. Fluorescence protein tagging of 7 previously uncharacterized TurboID-identified proteins showed that 6 were localized to a range of subpyrenoid regions. The resulting proxiome suggests new secondary functions for the pyrenoid in RNA-associated processes and redox sensitive iron-sulfur cluster metabolism. This developed pipeline opens the possibility of investigating a broad range of biological processes in Chlamydomonas plastid especially at a temporally resolved suborganellar resolution.

# An intimate view into the silica deposition vesicle of the model diatom *Thalassiosira pseudonana*

Kröger, N.<sup>1</sup>, Heintze, C.<sup>1</sup>, Babenko, I.<sup>1,2</sup> & Friedrich, B.M.<sup>2</sup>

<sup>1</sup>B CUBE, TU Dresden, Germany <sup>2</sup>Excellence Cluster Physics of Life, TU Dresden nils.kroeger@tu-dresden.de

The silica cell walls of diatoms are paradigms for the remarkable ability of organisms to generate inorganic materials with complex nano- and micropatterns that exceed by far the capabilities of current materials synthesis. During the past two decades, numerous candidate genes have been implicated in bio-morphogenesis of diatom silica, yet hardly any of them have been functionally characterized. Furthermore, general mechanistic models are lacking that would be able to explain how the encoded proteins can control silica morphogenesis from the nanometer scale up to scale of hundreds of micrometers. Recently, we have performed the first proteomics analysis of silica deposition vesicles (SDVs) which has enhanced the ability to identify genes involved in silica biogenesis with high confidence (https://doi.org/10.1073/pnas.221154911). Furthermore, we developed a method to visualize silica morphogenesis in diatoms with unprecedented detail (https://doi.org/10.1186/s42833020-00017-8). Combining these advances with targeted gene knockout allowed us to establish an experimental pipeline for the functional characterization of SDV proteins, and has started to provide invaluable information to develop models for silica morphogenesis mechanisms across the scales.

#### Aureochromes – Unique Photoreceptors in Diatoms

Kroth, P.G.<sup>1</sup>, Serif, M.<sup>1</sup>, Madhuri, S.<sup>1</sup>, Im, S.H.<sup>1</sup>, Lepetit, B.<sup>1</sup> & Wilhelm, C.<sup>3</sup>

<sup>1</sup>Department of Biology, University of Konstanz, Germany <sup>2</sup>Plant Physiology, Universität Leipzig, Germany Peter.Kroth@uni-konstanz.de Aureochromes (AUREOs) are a relatively novel and highly unusual type of blue-light photoreceptors in algae. They so far have only been found in stramenopile algae and have a very peculiar structure: In addition to a blue-light sensing flavin-binding LOV-domain, they possess a DNA-binding bZIP domain, that is typically found in transcription factors. Therefore, aureochromes can be considered as lightdriven transcription factors, allowing very fast responses by direct interaction with promoters. The pennate diatom *Phaeodactylum tricornutum* possesses four different aureochromes (PtAUREO1a,1b,1c,2), which are expressed differently throughout the day.

We have generated PtAureo knockout cell lines of the diatom *P. tricornutum*. PtAureo1a knockout mutants show a distinctive phenotype of a low capacity for Non Photochemical Quenching (NPQ), a prominent photoprotection mechanism in diatoms. We have confirmed the role of PtAUREO1a by genetic complementation studies in *P. tricornutum*. In transcriptomics experiments, we found that the knockout of PtAureo1a has a drastic impact on global gene expression of the algae, indicating that PtAUREO1a might function as a master switch for blue light induced responses. Furthermore, a knockout of PtAureo1a has an impact on the diel expression of other regulatory factors. Studies on the potential binding sites of Aureochromes via yeast-1-hybrid analyses indicate that PtAureo1a can regulate its own expression. We furthermore found that all four aureochromes may interact with each other, potentially allowing fine tuning of aureochrome responses. Here, I will combine an overview of the current knowledge on aureochromes with the latest results on functional aspects.

#### SYMPOSIUM 10 "Ecology of algal systems"

#### **KEYNOTE**

#### Ecological stoichiometry of phytoplankton disease

van de Waal, D.B.<sup>1</sup>, Peace, A.<sup>2</sup>, Clijsen, E.<sup>1</sup>, Kammers, K.<sup>1</sup>, Seabloom, E.W.<sup>3</sup>, Frenken, T.<sup>4</sup>, van den Wyngaert, S.<sup>5</sup> & Borer, E.T.<sup>3</sup>

<sup>1</sup>Netherlands Institute of Ecology (NIOO-KNAW), the Netherlands <sup>2</sup>Texas Tech University, United States of America <sup>3</sup>University of Minnesota, United States of America <sup>4</sup>HAS University of Applied Science, the Netherlands <sup>5</sup>University of Turku, Finland D.vandeWaal@nioo.knaw.nl

Primary producer hosts rely on nutrients for growth and defense against parasites. At the same time, parasites require host nutrients for their growth. Relationships between primary producer hosts and their parasites may be predicted by stoichiometric mismatches. At the same time, host defense also may be nutrient dependent, particularly on N, restricting the ability of cells to invest in their immunity under N limitation. We tested the effects of shifting host elemental ratios on interactions between host and parasite growth and host defense. To this end, we combined a literature survey, stoichiometric disease model, and laboratory experiments. Overall, our literature survey confirmed that relationship between hosts and parasites follows stoichiometrically predictable patterns, with reduced parasite performance under decreasing host N:C and P:C ratios. Our model, where host growth and defense, as well as parasite infections depend on N, revealed an optimum for infection rates at intermediate host N:C ratios. At lower host N:C ratios, the parasite became limited by nitrogen, while at high host N:C ratios infection was inhibited by host defense. We verified these findings with a green algal host infected by a fungal parasite (i.e., chytrid) exposed to a gradient in nitrogen availability. These findings were largely in line with the model outcome, showing reduced infections at both the lowest and highest nitrogen treatments. Overall, our findings demonstrate that nutrients can modulate host-parasite interactions via shifting host investment in growth and defense, providing a mechanistic base for understanding disease dynamics in changing nutrient conditions.

#### ORALS

### Interactive effects of warming and zooplankton grazing on a phytoplankton community of the Central Baltic Sea

#### Paul, C., Dutz, J. & Kremp, A.

*Leibniz-Institute for Baltic Sea Research Warnemünde, Germany* carolin.paul@io-warnemuende.de

In the Central Baltic Sea, Sea surface temperature (SST) in spring is already up to 2°C higher (2016) compared to the long-term average mean (1990-2016) and is predicted to increase up to 3.5°C until the end of this century. Warming has been suggested to be the main driver of the observed changes in

phytoplankton spring bloom biomass and community composition of the past decades in this area. We conducted a mesocosm experiment with a natural spring plankton community from the Bornholm Basin (Central Baltic Sea) under the combined effects of elevated temperature and excluding/including mesozooplankton (MZP) grazing. We hypothesized that warming will lead to changes in the phytoplankton biomass and species composition, induced by temperature-driven changes in the micro-and mesozooplankton community.

Results show that at the beginning of the experiment the phytoplankton community was dominated by the dinoflagellate *Peridinella catenata* and the mixotrophic ciliate *Mesodinium rubrum*, which typically follow the diatom bloom in this area. Elevated temperature lead to a faster drop-down of the dinoflagellate bloom, independent from zooplankton treatments. Analyses show that mesozooplankton grazing on MZP ciliates occur earlier in the spring bloom than previously observed and is enhanced at elevated temperature, changing the phytoplankton community size structure from large dinoflagellates to a dominance of smallsized pico- and nano phytoplankton.

Our results suggest that under future climate change the phytoplankton spring bloom community composition of the Central Baltic Sea will be altered due to temperature-induced changes in zooplankton abundances and trophic interactions.

### Selenium and marine algae – A trace micronutrient from an environmental perspective

Béziat, P.<sup>1,2</sup>, Le Bras, Z.<sup>1,2</sup>, Alcolombri, U.<sup>3</sup>, Bouchet, S.<sup>1,2</sup>, Tolu, J.<sup>1,2</sup>, Winkel, L.H.E.<sup>1,2</sup>

<sup>1</sup>Institute of Biogeochemistry and Pollutant Dynamics, ETH Zurich, Switzerland <sup>2</sup>Eawag, Swiss Federal Institute of Aquatic Science and Technology, Switzerland <sup>3</sup>Institute for Environmental Engineering, ETH Zurich, Switzerland pauline.beziat@usys.ethz.ch

The marine biosphere appears to be a centerpiece in global selenium (Se) cycling, channeling Se into the atmosphere via transformation of inorganic Se into volatile species such as dimethylselenide (DMSe). Correlations of chlorophyll and DMSe point to phytoplankton being involved in oceanic Se emissions, however a mechanistic understanding of their role in these DMSe emissions is missing to date. While it is assumed that the algal Se metabolism broadly follows that of S given the chemical similarities of these two elements, large differences in seawater concentrations might give rise to contrasting metabolic pathways: while S is in excess in seawater, Se is present at trace concentrations. Remarkably, these environmental Se levels are often much lower than what is required by laboratory phytoplankton cultures, many of which show strong obligate Se essentiality.

Here we present various analytical advancements to study Se in marine microbial communities, centered around the conundrum of why marine algae seem to volatilize a micronutrient present at concentrations so low, it might be limiting growth. We perform isotopic tracer experiments following the addition of inorganic selenite into co-cultures between *Emiliana huxleyi*, *Thalassiosira weissflogii* and the marine bacterium *Ruegeria pomeroyi*. Using a combination of LC-ICP-MS/MS and ESI-Orbitrap, we characterize uptake of Se into biomass and release of organic Se species back into seawater. We further follow the production of volatile Se species using thermal desorption (TD) GC-ICP-MS. Our results highlight the importance of moving from axenic cultures at high Se concentrations towards more natural conditions to further elucidate the role of algae in marine Se cycling.

# *Ostreopsis* spp. blooms in relation to macroalgal communities in the framework of regime shifts

Monserrat, M.<sup>1</sup>, Verdura, J.<sup>1</sup>, Asnaghi, V.<sup>2</sup>, Meroni, L.<sup>2</sup>, Chiantore, M.<sup>2</sup>, Lemée, R.<sup>3</sup>, Rossi, A.M.<sup>2</sup>, Priouzeau, F.<sup>1</sup>, Romero, G.<sup>1</sup> & Mangialajo, L.<sup>1</sup>

<sup>1</sup>Université Côte d'Azur, ECOSEAS, France <sup>2</sup>DiSTAV, Università degli Studi di Genova, Italy <sup>3</sup>Sorbonne Université, CNRS, Laboratoire d'Océanographie de Villefranche, France luisa.mangialajo@unice.fr

There has been an increasing occurrence of typically tropical benthic harmful algal blooms in temperate areas during the last decades, and ocean warming is expected to exacerbate this phenomenon in the near future. Blooms of *Ostreopsis* spp. are increasing in temperate regions around the world, mostly developing on macroalgal communities. Recent studies refer that blooms of *Ostreopsis* spp. could be associated with structurally less complex macroalgal communities (i.e. turfs *versus* forests), suggesting that the generalized phenomenon of habitat miniaturization, as the result of regime shifts in temperate ecosystems, could foster blooms of these species. With the objective to understand the role that marine vegetation could play in controlling the distribution and proliferation of *Ostreopsis* spp., its abundance during an annual bloom was quantified at different spatial scales in two localities of the Ligurian Sea (French Riviera, France and Liguria region, Italy). Samplings were performed on different macroalgal species and communities, including artificial substrates. The results obtained highlight high abundances of *Ostreopsis* spp. on the different substrates and suggest a higher suitability of less complex macroalgal communities in hosting *Ostreopsis* spp. The high variability detected could potentially hinder further and more detailed patterns of biotic substrate preference.

# Investigating the molecular mechanism of diatom's adhesion using metabolomics to innovate antifouling materials

Castaldi, A.<sup>1,4</sup>, Le Moyec, L.<sup>1,2</sup>, Triba, M.N.<sup>3</sup>, Blond, A.<sup>1</sup>, Marie, A.<sup>1</sup>, Le Pennec, G.<sup>4</sup> & Bourguet-Kondracki, M-L.<sup>1</sup>

<sup>1</sup>Laboratoire Molécules de Communication et Adaptation des Micro-organisms UMR 7245 CNRS, Muséum National d'Histoire Naturelle, 57 rue Cuvier (C.P. 54), 75005 Paris, France

<sup>2</sup>Université d'Evry Val d'Essonne, Université Paris Saclay, Boulevard F. Mitterrand, 91025 Evry, France

<sup>3</sup>Laboratoire UFR Santé, Médecine et Biologie Humaine CSPBAT- UMR 7244, Université Sorbonne Paris Nord, 74 rue Marcel Cachin, 93017 Bobigny Cedex, France

<sup>4</sup>Laboratoire de Biotechnologie et Chimie Marines, Université Bretagne Sud, EMR CNRS 6076, IUEM, 56100 Lorient, France

andrea.castaldi1@mnhn.fr

Diatoms are with bacteria the first players in the colonization of surfaces, forming a tough and resilient biofilm, leading to fouling. Biofouling is a phenomenon inherent to all submerged structures: ship hulls, aquaculture equipment, marine energy installations, which forces the implementation of costly preventive and curative measures. Societal, environmental and regulatory pressures are severely restricting the use of toxic compounds present in antifouling formulations. One promising strategy to combat fouling is based on the surface properties of materials and/or coatings (low energy, roughness), which consists in reducing forces between organisms and surfaces. Another one could be to better understand the biofilm installation on surfaces. However, diatoms have not been extensively studied and

knowledge of the processes involved in their attachment remains -poorly understood. The few publications available underline the importance of better understanding the role of different factors that can be particularly decisive for diatom adhesion: surface topography, conditioning by exoproducts (exopolysaccharides, communication molecules).

In order to explore the adhesion strategies of diatoms, we have studied at the molecular level how the diatoms *Phaeodactylum tricornutum* and *Cylindrotheca closterium* modify their metabolism to perform their adhesion according to the substrate encountered (glass, polystyrene, polydimethylsiloxane). Mass spectrometry and NMR spectroscopy were used to analyze five replicates of each culture on the three substrates plus one planktonic culture. Thanks to multivariate statistical analyses, we found that the metabolome of these two diatoms varies according to the support and the adhesion capacity of the diatom strains, opening up promising perspectives for antifouling targets.

### Effects of nutrient limitation on survival, infectivity, metabolism and swimming behaviour of *Amoebophrya ceratii* dinospores

Romero Rodríguez, I., Labry, C., Le Gac, M., Rabiller, E., Schmitt, S. & Jauzein, C.

IFREMER DYNECO Pelagos, France Irene.Romero.Rodriguez@ifremer.fr

Among marine planktonic organisms, *Amoebophrya* species are specialized parasites of phytoplankton. They are involved in the biological control of blooms of various dinoflagellate species, including toxic ones. Little is known about this organism free-living stage, the dinospore, including its survival and infection strategies in the marine environment. More specifically, nutritive strategies of dinospores are currently unknown. Whether they rely on intracellular stocks coming from the host or on the uptake of nutrients from the media is yet to be determined.

A study was carried out in order to test if dinospores are able to take up nutritive sources from the extracellular medium in order to survive and support metabolic costs of swimming and infection. Freshly released dinospores were maintained during a week and a half under either replete culture condition (f/2 medium) or limited ones. Five limiting conditions were tested over two sets of experimentation: depletion of phosphorous, vitamins, metals, of these three elements, as well as using the host lysate as the sole nutritive source. Survival of *Amoebophrya* dinospores was monitored by flow cytometry twice a day during seven days. Nutritive strategies were characterized at 40, 112 and 160h, by measuring <sup>33</sup>PO<sub>4</sub><sup>3-</sup>-uptake, cellular quotas of ATP, phosphorous, and dissolved organic phosphorous concentrations. Infection success was estimated simultaneously. Swimming behaviors were determined at 40 and 112h. Our results prove that *Amoebophrya certaii* dinospores take up dissolved phosphorous from the environment. In addition, they unexpectedly showed that survival is better in depleted media than in enriched media.

### Adaption of a non-native *Vaucheria* species to different light conditions in the Wadden Sea (north-east Atlantic)

Steinberg, R.<sup>1</sup>, Bischof, K.<sup>1</sup> & Buschbaum, C.<sup>2</sup>

<sup>1</sup>University of Bremen, Leobener Str. NW2, D-28359 Bremen, Germany <sup>2</sup>Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research, Wadden Sea Station Sylt, Hafenstrasse 43, D-25992 List, Germany rsteinbe@uni-bremen.de

#### TUESDAY 22<sup>nd</sup> August 2023 Room « Tali friz »

Worldwide coastal areas are affected by an increasing number of introduced species. This is also the case for the European Wadden Sea (south-eastern North Sea), which represents the largest coherent sedimentary tidal coast of the world. Here, extensive mats of the filamentous xanthophyceae Vaucheria sp. have been recently found in the intertidal as well as in the shallow subtidal zone. Genetic studies revealed that this Vaucheria-species is a non-native species originating from overseas. It shows effective adaptive mechanisms to abiotic conditions in the respective tidal zone where it has successfully established. Vaucheria sp. have been found in an entirely subtidal as well as in intertidal environments, where they are intermittently exposed to high light during low tide. Photosynthesis vs. irradiance measurements indicate that the intertidal Vaucheria specimens have adapted to cope with intense light levels by seasonally altering their pigment composition, i.e., by concentrations ratio of chlorophyll to the xanthophylls diadinoxanthin and diatoxanthin. The xanthophylls protect the photosynthetic system of Vaucheria sp. against high light intensity. The intertidal Vaucheria specimens have a three times higher chlorophyll/xanthophyll ratio in November than in July and September due to the low xanthophyll concentration, whereas subtidal Vaucheria specimens, by contrast, have a constant pigment concentration ratio in July, September and November. These different temporal pigment patterns of intertidal and subtidal Vaucheria specimens demonstrate the species' ability to adapt to different environmental conditions, which may contribute to its rapid proliferation in the Wadden Sea.

#### **KEYNOTE**

#### Chemical signaling in the algal holobiont

#### Vallet, M.<sup>1,2</sup>

 <sup>1</sup>Research Group Plankton Community Interaction, Max Planck Institute for Chemical Ecology, Jena, Germany
 <sup>2</sup>Bioorganic Analytics, Institute for Inorganic and Analytical Chemistry, Friedrich Schiller University Jena, Germany
 mvallet@ice.mpg.de

In aquatic ecosystems, algae serve as primary producers of the food web and live in complex interactions with microorganisms. The algal host and its cohort of symbionts create a biomolecular network with exchanged chemical cues, coined as the algal holobiont. The algal host cells are using specialized metabolic and behavioral strategies to thrive despite biotic and abiotic stresses, such as pathogens and heat waves. Chemical signaling is involved in the mediation of these host-symbiont interactions and can be investigated by state-of-art metabolomics. To date, few studies have focused on the algal holobiont and the chemical substances involved in host cell regulation.

In my talk, I will give insights into the chemical mediation of microbes associated with seaweeds and phytoplankton. I will show how marine oomycete parasites can manipulate their host metabolism to induce bloom termination. Further, using a pathosystem parasite model in seaweed, we identified novel chemical cues from fungal symbionts that are involved in the defense of the algal holobiont against infectious pathogens. We also developed a novel workflow to study parasitic diseases using single-cell metabolomics and identify metabolites associated with infection in algae. By using high-resolution mass spectrometry, we are now able to define the chemical mediation of the algal holobiont and provide insights into the functioning of aquatic communities.

#### ORALS

# Geographical variability of physiological responses of *Ascophyllum nodosum* to realistic simulations of intertidal conditions in a selfcontained autonomous system

Pereira, L.F.<sup>1,2,3</sup>, Monteiro, C.<sup>1,3</sup>, Arenas, F.<sup>4</sup>, Seabra, R.<sup>1,3</sup>, Lima, F.P.<sup>1,3</sup>

<sup>1</sup>CIBIO, Research Centre in Biodiversity and Genetic Resources - InBIO Associate Laboratory, Campus of Vairão, University of Porto, Vairão, Portugal

<sup>2</sup>Department of Biology, Faculty of Sciences, University of Porto, Porto, Portugal

<sup>3</sup>BIOPOLIS Program in Genomics, Biodiversity and Land Planning, CIBIO, Campus of Vairão, Vairão, Portugal

<sup>4</sup>CIIMAR, Interdisciplinary Centre of Marine and Environmental Research of University of Porto, Avenida General Norton de Matos, Matosinhos, Portugal

luisferreirapereira@gmail.com

Predicting the biological effects of climate change is still a major challenge today. To date, most studies have yet to properly incorporate the notions that a) climate change is not happening uniformly across the globe, and that b) there is substantial variability in the way individuals and/or populations of a given species respond to such changes In this work we tackled both issues by (i) employing a new generation of temperature data-loggers to measure temperature variability at the scale of the studied organisms, (ii) using data from the temperature dataloggers to perform realistic experiments in the lab, (iii) evaluating how populations originating from distinct locations across the span of the species range cope with similar levels of thermal stress.

We focused on the intertidal macroalgae *Ascophyllum nodosum*, a canopy-forming and coldadapted species whose distribution in Europe ranges from Svalbard to NW Iberia. We collected specimens from 10 populations from Norway, NW France and NW Iberia, including the southernmost population in north Portugal. Experiments included realistic simulation of photoperiod, and high and low tide temperatures (by carefully manipulating water temperature as well as simulating solar heating with IR lamps). This was a technical challenge per se, as it required the development of new environmental simulation system. Organism performance was evaluated through the quantification of mortality, growth, photosynthesis efficiency and respirometry. This study highlights the need to incorporate intraspecific variability in the responses to environmental change in order to understand and correctly forecast future changes in species distributions.

#### Peak fucoid canopy photosynthesis in space and time

#### Johnson, M.P.

Ryan Institute and School of Natural Sciences, University of Galway, Ireland mark.johnson@universityofgalway.ie

Recent global estimates of net photosynthesis by algal canopies include high rates for intertidal species such as fucoids. However, the data behind such estimates are very variable, leaving uncertainties about the amount of net production by canopies. Models can help synthesize a deeper understanding of canopy photosynthesis, identifying features such as the biomass at which peak production occurs. A recent canopy photosynthesis model suggests that fucoid canopies may be close to biomass levels where peak production occurs. This peak will vary with the light climate, meaning that the optimum biomass will vary on latitudinal gradients. Furthermore, seasonal variation in biomass, with lower levels in winter, is

#### TUESDAY 22<sup>nd</sup> August 2023 Room « Tali friz »

widely observed. Lower winter biomass may maintain positive net canopy production in the face of lower light levels. Without a lower canopy biomass in winter, canopies may approach situations where tissue respiration exceeds photosynthesis. Whether lower canopy biomass in winter can be considered a truly deciduous pattern depends on the costs for fronds staying intact as light levels fall. Further characterization of canopy biomass in space and time is needed to understand the production by macroalgal canopies and the contribution of seasonally lost biomass to ecosystem function.

### *Prymnesium parvum,* a bloom-forming haptophyte that use toxins to mediate food uptake, especially under P-limitation

Boucher, C.<sup>1</sup>, André, J.<sup>1</sup>, Sibat, M.<sup>1</sup>, Réveillon, D.<sup>1</sup>, Lacour, T.<sup>1</sup>, Hansen, P.J.<sup>2</sup> & Mairet, F<sup>1</sup>

<sup>1</sup>Ifremer, Unit PHYTOX, Nantes, France

<sup>2</sup>University of Copenhagen, Department of Biology, Marine Biological Section, Denmark clemence.boucher@ifremer.fr

The haptophyte *Prymnesium parvum* is one of the most widespread microalgae associated with fish kills in the world. It produces lytic toxins, called prymnesins, which act on cell membranes of target cells; thus this alga not only kills fish but may impacts the entire ecosystem. It is a mixotrophic alga, which utilizes its toxins to immobilize and catch prey, allowing it to exploit organic nutrients (N and P).

Using *P. parvum* (CCAP 946/6 strain) as a model, experiments were conducted to better understand the external factors leading to an increase of growth rate, phagotrophic activity and toxicity (intra- and extracellular). P-deficient *P. parvum* cultures were grown with or without addition of phosphorus (P) as either inorganic nutrient and/or algal prey (i.e. the cryptophyte *Teleaulax amphioxeia*). Samples for toxin analyses were taken at three time intervals (3, 7 and 9 days). Detection and quantification of the phagotrophic activity and toxin content were performed using flow cytometry and high resolution mass spectrometry (LCHRMS), respectively. In addition, to assess the lytic activity of *P. parvum*, microalgal bioassays were performed on the supernatant towards *T. amphioxeia*.

Our results show that CCAP 946/6 strain produces A-type prymnesins (1 and 2), with a higher cell prymnesin content in P-deficient cultures compared to P-sufficient cultures. Thus, *P. parvum* combines the production of lytic toxins and phagotrophy to improve its growth under P limitation, explaining why it has such a big success in estuaries during summer periods.

# Selective feeding of constitutive mixotrophic dinoflagellates in response on different quality prey

Blais, C.<sup>1,2</sup>, Moriceau, B.<sup>1</sup> & Maguer, J.F.<sup>1</sup>

<sup>1</sup>Univ Brest, CNRS, IRD, Ifremer, UMR 6539, LEMAR, Plouzane, France <sup>2</sup>Takuvik International Research Laboratory (IRL 3376), ULaval – CNRS, Biology department, Laval University, Quebec, Canada clemence.blais@univ-brest.fr

Mixotrophic is a nutritional strategy that can combine different trophic modes in a single organism (Mitra et al., 2014, 2016). This allows the organism to use different carbon resources, a capability often described as an advantage in climate change. However, limited knowledge of the mechanisms that determine the intensity of mixotrophy in organism is known. In our study, we focused on constitutive mixotrophs (i.e. continuously autotrophs and in some conditions to be identified able to use

#### TUESDAY 22<sup>nd</sup> August 2023 Room « Tali friz »

phagotrophy). But, are constitutive mixotrophic dinoflagellate able to select their prey ? Or do they just eat prey randomly depending on their encounter rate ?

To answer this question, we tested different prey qualities (size, coloration, viability, silicification) on the predation ability of two dinoflagellates (*Alexandrium tamarense* and *Heterocapsa triquetra*). For the first time, we show that the environment is not the only trigger for the mixotrophy, the type or quality of the prey has an important impact on feeding selection. Three elements are insignificant for prey selection: silicification, viability and size. Indeed, the dinoflagellates tested ingested the same amount of carbon whether the prey was a cyanobacteria or a diatom. No significant difference was measured in the ingestion rates of dinoflagellates on silicified or non-silicified cyanobacteria and on dead cyanobacteria compared to live cyanobacteria. The ingestion rates were constant and in the order of 1 prey/dinoflagellate/h.

Althought we didn't measure a difference between live and dead prey, we did observed a change in the predator's ingestion rate when the prey was stressed. The different coloration of cyanobacteria obtained with different light regimes had an impact on the ingestion rate of dinoflagellate. Ingest rates can even decrease by 80 %. We highlight that the carbon composition of these different prey isn't significant. However, these different prey do not have the same fluorescence emission response allowing us to hypothesis that they may be at different stages of stress.

Mixotrophic organism thus have a singular function in their relationship with their prey, they use the organic matter of prey according to their physiological quality.

#### Cornered by Turfs: The Alarming Decline of Forest-like Macroalgal Communities in the South-Eastern Bay of Biscay

Arriaga, O.<sup>1</sup>, Wawrzynkowski, P.<sup>2</sup>, Gorostiaga, J.M.<sup>1</sup>, Muguerza, N.<sup>1</sup>, Díez, I.<sup>1</sup>, González, J.<sup>1</sup>, Quintano, E.<sup>1</sup> & Becerro, M.A.<sup>2</sup>

<sup>1</sup>Department of Plant Biology and Ecology, Faculty of Science and Technology & Research Centre for Experimental Marine Biology and Biotechnology PIE-UPV/EHU, University of the Basque Country (UPV/EHU), Bilbao, SPAIN.

<sup>2</sup>The BITES lab, Center for Advanced Studies of Blanes (CEAB-CSIC), Blanes (Girona), SPAIN olatz.arriaga@ehu.eus

Our study highlights the significant decline in forest-like macroalgal communities in the shallow subtidal vegetation off the south-eastern coast of the Bay of Biscay. We studied 19 locations spread over 100 km off the Basque Country coast in 1991, 2008, 2013, and 2021 and documented rapid degradation in most of the studied locations. We found that macroalgal forests shifted towards structurally less complex communities dominated by turf-forming species. The shift has led to a significant increase in taxa richness and diversity, mainly due to the development of opportunistic and morphologically simple forms. The widespread loss of these communities has also led to shifts in ecosystem services linked to reduced biomass and productivity. The prevalence of the new turf communities seems to represent an alternate stable state for the temperate macroalgal communities in the area.

Recent community changes have failed to revert to forest-like communities, and changes over the last years were minor variations of the turf community theme. Moreover, the most pronounced increase in calcareous species and morphologically simple forms has occurred over the last period, from 2013 to 2021. However, some coastal areas have remained almost unchanged since 2008, representing refuge locations for those canopy species that are being replaced in adjacent locations.

Although additional factors play a role in the transition from forests to turfs, we seem to be experiencing the loss of temperate macroalgal forests due to climate change.

### What are you doing here? A surprising beta-porphyranase activity in a carrageenophyte red alga

Manat, G.<sup>1</sup>, Fanuel, M.<sup>2,3</sup>, Jouanneau, D.<sup>1</sup>, Jam, M.<sup>1</sup>, Mac-Bear, J.<sup>2,3</sup>, Rogniaux, H.<sup>2,3</sup>, Mora, T.<sup>1</sup>, Larocque, R.<sup>4</sup>, Lipinska, A.<sup>1</sup>, Czjzek, M.<sup>1</sup>, Ropartz, D.<sup>2,3</sup> & Ficko-Blean, E.<sup>1</sup>

<sup>1</sup>CNRS, Integrative Biology of Marine Models (LBI2M), Station Biologique de Roscoff (SBR), Sorbonne Université, Roscoff, France
 <sup>2</sup>INRAE, UR BIA, Nantes, France
 <sup>3</sup>INRAE, BIBS Facility, Nantes, France
 <sup>4</sup>CNRS, FR 2424, Station Biologique de Roscoff, Sorbonne Université, Roscoff, France
 eficko-blean@sb-roscoff.fr

The extracellular matrix (ECM) is integral to the biology of algae. Carrageenophyte red algae, including the model red macroalga *Chondrus crispus*, have the complex sulfated polysaccharide carrageenan as a main component of their matrices. We identified a family 16 glycoside hydrolase, CcGH16-3, as a potential carrageenan-modifying target from *C. crispus* as its gene is differentially expressed between the algal life cycle stages which have different carrageenan compositions.

A GH16 family phylogeny shows the CcGH16-3 enzyme is related to bacterial carrageenases and porphyranases and is likely due a horizontal gene transfer. Unexpectedly, after biochemical characterization, we discovered it to be a beta-porphyranase. Porphyran is not a known component of the *C. crispus* ECM; however, it is a known component of other red algal matrices. Using aqueously extracted porphyran, we investigated the kinetics for CcGH16-3. We undertook a structural subsite dissection using specialized mass spectrometry which limits sulfate lability. Our analysis provides a model of the active site and defines fine enzymatic specificity. This provides the first characterization of an algal beta-porphyranase.

CcGH16-3 is one of a handful of algal enzymes characterized with a specificity for a motif unknown to be found in their own ECM. The enzyme may function to hydrolyze minority porphyran motifs not yet identified in the carrageenophyte host matrix, provide defense against red algal pathogens or provide a competitive advantage in niche colonization. Overall, this is a significant biochemical advance to understanding the recognition of biopolymers unique to marine ecosystems by complex multicellular organisms.

### Global survey of vitamin B<sub>12</sub>-related genes in phytoplankton reveals contrasting metabolic abilities between communities

Nef, C.<sup>1,2</sup>, Dorrell, R.<sup>1,3</sup>, Smith, A.<sup>3</sup> & Bowler, C.<sup>1,2</sup>

<sup>1</sup>Institut de Biologie de l'École Normale Supérieure (IBENS), École Normale Supérieure, CNRS, INSERM, PSL Université Paris, 75005 Paris, France.

<sup>2</sup>*Research Federation for the study of Global Ocean Systems Ecology and Evolution, FR2022/Tara Oceans, Paris, France.* 

<sup>3</sup>Department of Plant Sciences, University of Cambridge, Downing Street, Cambridge CB23EA, United Kingdom

charlotte.nef@bio.ens.psl.eu

Within marine plankton communities, phytoplankton and prokaryotes interact through complex exchanges of secreted bioactive compounds. Among the elements that may be traded are B-vitamins,

complex organic molecules required in small amounts, which act as antioxidants, ligands and/or enzymatic cofactors. Vitamin  $B_{12}$  (cobalamin) is required by many algal species, but it is synthesized by a subset of bacteria and archaea only. The metabolic dependency towards cobalamin relates to the presence of a  $B_{12}$ -dependent methionine synthase enzyme (METH) and the absence of a zinc-dependent isoform (METE).  $B_{12}$  limits phytoplankton community development in almost every oceanic region in which amendment experiments have been undertaken, suggesting that its availability may have important ecological implications.

With the aim of disentangling phytoplankton capability to mitigate cobalamin limitation in the oceans, the present study investigated the distribution of the different methionine synthase genes, together with functions potentially involved in cobalamin acquisition, scavenging and remodelling within an ecological context, across more than 1,500 algal genomes and transcriptomes and using the global-scale samples from the *Tara* Oceans expeditions. Our results highlight a wide variability of strategies in phytoplankton communities, while delineating more constrained phytoplankton groups with obligate  $B_{12}$  dependence, and suggest potential community inter-replacement depending on the oceanic regions. This study represents, to our knowledge, the first pole-to-pole catalogue of phytoplankton metabolic strategies regarding vitamin  $B_{12}$  availability.

### Coastal peatland water stimulates growth of Baltic Sea and peatland benthic diatoms

#### Prelle, L.R. & Karsten, U.

Institute of Biological Science, Applied Ecology and Phycology, University Rostock, Rostock, Germany lara.prelle@uni-rostock.de

Shallow coastal waters are unique ecosystems which are strongly involved in the exchange processes along a marine-terrestrial gradient. Along the coastal zones of the southern Baltic Sea adjacent terrestrial habitats, such as peatlands, are often separated by dunes and dikes from the Baltic Sea preventing natural exchange processes of these two ecosystems. With the ongoing climate change, the immediate link to the sea level rise and increasing storm surge events as well as increasing renaturation of coastal peatlands, the amount of naturally or artificially removed dunes and dikes is increasing. The resulting increased exchange of both ecosystems leads to inhabiting organisms, such as benthic diatoms, to suddenly facing new environmental settings including changes in salinity, light availability and nutrient conditions with potential consequences for photosynthesis and growth.

Using *in-vivo* fluorimetry, growth response of five Baltic Sea and peatland benthic diatoms to ten different growth media (based on Baltic Sea water and/or peatland water with varying additives) clearly displayed a growth stimulation in peatland-water based media. In addition, growth of two Baltic Sea species was shown to be inhibited in the freshwater peatland media, indicating for salinity to be an important co-parameter for species-specific growth during the mixing of both ecosystems. While measurements of species-specific photosynthetic rates with increasing photon fluence rates (0-1400  $\mu$ mol photons m<sup>-2</sup> s<sup>-1</sup>, recorded at 20°C) in two selected media illustrated a high photo-physiological plasticity in the five diatom strains, only one species displayed a difference in photosynthetic performance depending on the growth medium.

### Mediterranean deep water kelp *Laminaria rodriguezii* is living at optimum temperatures for growth but at the edge of light

Pageault, L.<sup>1,2</sup>, Wagner, A.<sup>1</sup>, Thibaut, T.<sup>2</sup> & Bartsch, I.<sup>1</sup>

<sup>1</sup>Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research, Germany <sup>2</sup>Aix Marseille University, Mediterranean Institute of Oceanography (MIO), UM 110, Marseille, France inka.bartsch@awi.de

The Mediterranean deep-water kelp *Laminaria rodriguezii*, develops large populations of ecological importance, at depth >50-70m. Not much is known about its ecology or physiological performance. For the first time we managed to get fertile material from the Banc de Magaud, Provence, France, and isolated clonal gametophytes. From these, we cultivated juvenile sporophytes of *L. rodriguezii* in order to investigate growth in a temperature and an irradiance gradient (n=5). In addition we determined the photosynthetic performance of material cultivated in the irradiance gradient at 15°C (n=3).

Juvenile sporophytes grew optimally between 10 and 15°C, even survived 0°C, but became severely hampered at 23°C, and died at 25°C after 14 days. At 15°C growth was optimal in a wide range of irradiances (12-200  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>), but sporophytes still grew slowly even in very low light (1  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>). Similarly, gross oxygen production GOP showed a wide maximum between 24-200  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup> after 14 days of cultivation, and GOP was very low but still positive at lowest irradiances (1  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>). Photosynthetic irradiance curves and derived parameters revealed that *L. rodriguezii* maintains a high photosynthetic efficiency ( $\alpha$ ), a low saturating irradiance I<sub>k</sub> and high chl*a* levels at low irradiances, indicating its ability to acclimatize to low irradiances. Available *in situ* temperature data from the Banc de Magaud (13°C) suggest that *L. rodriguezii* thrives at optimal temperatures but sub-optimal low irradiances. Its upper distribution limit possibly is rather restricted by high temperatures than irradiance stress or a combination of both.

### Sexual selection in seaweed ? The case of the red alga Gracilaria gracilis

Valero, M.<sup>1</sup>, Lavaut, E.<sup>1</sup>, Mauger, S.<sup>1</sup>, Guillemin, M.L.<sup>1,2</sup>, Destombe, C.<sup>1</sup> & Dufay, M.<sup>3</sup>

<sup>1</sup>IRL EBEA 3614, Evolutionary Biology and Ecology of Algae, CNRS, Sorbonne Université, Pontificia Universidad Católica de Chile, Universidad Austral de Chile, Station Biologique de Roscoff, CS 90074, Place Georges Teissier, 29688 Roscoff cedex

<sup>2</sup>Instituto de Ciencias Ambientales y Evolutivas, Facultad de Ciencias, Universidad Austral de Chile, Valdivia, Chile

<sup>3</sup>*CEFE, Univ Montpellier, CNRS, EPHE, IRD, Montpellier, France* valero@sb-roscoff.fr

Sexual selection is a fundamental force in biology. Sexual selection theory postulates that anisogamy should lead to strong competition among males for the access to females. Quantification of sexual selection in both sexes has been achieved in a large number of animal species, by using metrics derived from the three Bateman's principles: variance in (i) reproductive and in (ii) mating success is larger for males than females and (iii) relationship between reproductive success and mating success (Bateman's gradient) is also stronger for males. However, these metrics have only been estimated and compared between males and females in three angiosperm species and never in seaweed.

#### TUESDAY 22<sup>nd</sup> August 2023 Room « Tali friz »

Here we focus on the oogamous haploid-diploid red alga *Gracilaria gracilis* in which previous studies have demonstrated non-random mating and animal-mediated transport of spermatia. We estimated mating and reproductive success using paternity analyses in a natural population where up to 92% of fertilizations occurred between partners of this population. The results show that the variance in mating success is significantly greater in males than in females and that the Bateman gradient is positive only in males suggesting a greater opportunity for sexual selection in males than in females in this species in which there is no marked sexual dimorphism.

The investigation of Bateman principles in species outside of the clades classically studied for sexual selection should help in understanding how and when anisogamy triggers differences in the extent of sexual selection between the sexes, and when these differences can translate in the evolution of sexual dimorphism.

# SYMPOSIUM 08 "Algal photosynthesis, carbon fixation and respiration"

#### **KEYNOTE**

#### CO<sub>2</sub>-concentrating mechanism in marine diatoms

Matsuda, Y. & Shimakawa, G.

School of Biological and Environmental Sciences, Kwansei Gakuin University, Japan yusuke@kwansei.ac.jp

Marine diatom is one of major primary producers in the oceans and they perform photosynthesis of up to 20% of the annual global CO<sub>2</sub> fixation. Diatom photosynthesis in seawater requires biophysical CO<sub>2</sub>-concentrating mechanism (CCM) which is sustained by active transport of  $HCO_3^-$  from the bulk medium across plasma membrane and chloroplast envelope. Due to the CCM, diatoms can perform high Ci affinity photosynthesis under limited CO<sub>2</sub> conditions with abundant  $HCO_3^-$  in seawater. Accumulated Ci in the stroma is maintained as  $HCO_3^-$  and CO<sub>2</sub> is supposed to be generated from this Ci stock only at the proximity of Rubisco. Inner plastidal structure that enables such highly localized formation of very slippery CO<sub>2</sub> molecule is now believed to be constituted by a liquid-liquid phase separation droplet within the stroma, the pyrenoid. The diatom pyrenoid holds double layered thylakoid tubes penetrating the center of the pyrenoid and this peculiar thylakoid system holds specific theta type carbonic anhydrase enzyme in the lumen. The model how diatom pyrenoid and chloroplast perform the biophysical CCM will be discussed with newly discovered diatom chloroplastic factors.

#### ORALS

# Bestrophin-like proteins play a key role in HCO<sub>3</sub><sup>-</sup> transport in the *Chlamydomonas* CO<sub>2</sub> concentrating mechanism

Walker, C.E.<sup>1</sup>, Adler, L.<sup>2</sup>, Lefoulon, C.<sup>3</sup>, Lau, J.<sup>1</sup>, Girr, P.<sup>1</sup>, Blatt, M.<sup>3</sup>, Peltier, G.<sup>4</sup>, McCormick, A.<sup>2</sup> & Mackinder, L.C.M.<sup>1</sup>

<sup>1</sup>University of York, UK <sup>2</sup>University of Edinburgh, UK <sup>3</sup>University of Glasgow, UK <sup>4</sup>Institut de Biosciences et Biotechnologies Aix-Marseille, CEA Cadarache, SaintPaul-lez-Durance, France charlotte.walker@york.ac.uk

Many algae operate biophysical  $CO_2$  concentrating mechanisms (CCMs) to enhance their photosynthetic output by delivering concentrated  $CO_2$  to Rubisco packaged in a specialised compartment called the pyrenoid. The current model of the *Chlamydomonas* CCM proposes that three bestrophin-like proteins (BST1, 2 and 3) play a key role by facilitating  $HCO_3^-$  transport across the thylakoid membrane, from the chloroplast stroma into the lumen. Once in the lumen, the  $HCO_3^-$  is available for dehydration by a carbonic anhydrase (CAH3) to form  $CO_2$ , which subsequently diffuses out of the thylakoid tubules that

TUESDAY 22<sup>nd</sup> August 2023 Room « Tali melen »

traverse the pyrenoid for fixation by Rubisco in the pyrenoid. However, to date, direct evidence of  $HCO_3^-$  transport by BST1-3 is lacking and the functional importance of individual BSTs in unknown. In this study we use CRISPR/Cas9 knock-out mutant characterisation, and electrophysiology in a *Xenopus* heterologous expression system to significantly aid our understanding of the function of these proteins and their importance in the *Chlamydomonas* CCM. As modelling proposes that a thylakoid localised  $HCO_3^-$  channel will be key to the successful introduction of a CCM into C3 plants, the generated data in this study is helping guide engineering efforts in higher plant systems.

### Bestrophin-knock out gave an impaired CCM phenotype in marine diatom, *Phaeodactylum tricornutum*

Nigishi, M., Yamagishi, K., Amano, R., Ito, S., Shimakawa, G. & Matsuda, Y.

Marine Bioscience, School of Biological and Environmental Sciences, Kwansei Gakuin University, Japan gzt54782@kwansei.ac.jp

Dissolved  $CO_2$  concentration is low is easily hydrated into  $HCO_3^-$  in alkaline seawater, marine diatoms operate  $CO_2$ -concentrating mechanism (CCM) to increase  $CO_2$  around Rubisco. In the diatom chloroplast, there are two types of thylakoid membranes, girdle lamellae (GL) and pyrenoids penetrating thylakoid membrane (PPTM).  $HCO_3^-$  is dehydrated to  $CO_2$  by  $\theta$ -type carbonic anhydrase in the lumen of PPTM providing  $CO_2$  to the Rubisco in the pyrenoid. Bestrophin (Best) is a candidate for  $HCO_3^-$  transporter at both thylakoid membranes, GL and PPTM.

In this study, we first analyzed the environmental response of Bests expressions at transcript and protein levels in marine diatoms, *Phaeodactylum tricornutum* and *Thalassiosira pseudonana* to estimate their roles in photosynthesis and CCM. PtBest1,2 located at GL showed increases in mRNA and protein in the atmospheric low CO<sub>2</sub> (LC) and CO<sub>2</sub> deprived very low CO<sub>2</sub> (VLC). Expression was further enhanced in cells grown under high light. PtBest3 was localized at PPTM, increased in mRNA level in LC and VLC. On the other hand, TpBest1, 2 were localized probably at PPTM. They showed a constitutive level of mRNA, while increased in protein levels in LC and VLC relative to HC, but the protein decreased in high light grown cells. Genome-editing knock out of PtBest1 by CRISPR-Cas9 nickase resulted in very low photosynthetic DIC affinities, which were comparable to that of WT grown in HC. The data indicates that PtBest1 is a critical factor for the CCM operation in *P. tricornutum*.

### Growth patterns and carbon fluxes: investigation of *Ulva* spp. primary metabolism using <sup>13</sup>CO<sub>2</sub>-labelling.

Simon, C.<sup>1</sup>, Perez de Souza, L.<sup>2</sup>, McHale, M.<sup>1</sup>, Inaba, M.<sup>1</sup>, Fernie, A.R.<sup>2</sup>, Sulpice, R.<sup>1</sup>

<sup>1</sup>Plant Systems Plant Systems Biology Laboratory, Ryan Institute & Marei Centre for Marine, Climate and Energy, School of Biological & Chemical Sciences, University of Galway, H91 TK33 Galway, Ireland. <sup>2</sup>Max Planck Institute of Molecular Plant Physiology, Am Müehlenberg 1, Golm, 14476 Potsdam, Germany. c.simon2@nuigalway.ie

Fast growing *Ulva* species are excellent candidates for the provision of high-quality algal biomass. *Ulva* spp. possess a unique diurnal growth pattern compared to terrestrial plants, with higher rates of tissue expansion during the night than during light periods. However, this observation is from image analysis and the variation in biomass composition has not been described. To assess the nocturnal accumulation of structural compounds and determine which carbon sources support this growth, we investigated the

primary metabolism of two *Ulva* species, *Ulva australis* and *Ulva compressa*, using <sup>13</sup>CO<sub>2</sub> isotope labelling. Gas chromatography-mass spectrometry (GC-MS) has been used to monitor the <sup>13</sup>C distribution in structural and non-structural compounds over a full diurnal cycle. The experiment consisted of a 12h daytime <sup>13</sup>CO<sub>2</sub> pulse, followed by a 12h chase in the dark, with samples collected every 4 hours over 24 hours. For both species, our analysis identified strong <sup>13</sup>C enrichment during the pulse phase, with 4 hours being the time required to reach steady state and a maximum <sup>13</sup>C ratio of 35% for many metabolites including some amino acids and soluble sugars. On average the pool of quantified soluble metabolites was labelled at 23% at 4 hours of pulse, reaching 25% at the end of the pulse and decrease to 18% at the end of the chase. Quantitatively, we determined that proline was the most abundant soluble metabolite, with a maximum of 1.0 mg.g DW<sup>-1</sup>, and showed a maximum labelling of 20%. Altogether our data indicates very high metabolic activity in *Ulva* spp., which could eventually explain its high growth rates.

### Local adaptation across multiple life stages and populations: implications for Kelps resilience to ongoing climate change

Fernández, P.A.<sup>1</sup>, Villegas, K.<sup>2</sup>, Palma, G.<sup>2</sup>, Torres, R.<sup>3</sup>, Pérez-Santos, I.<sup>2,3,4</sup> & Buschmann, A.H.<sup>1</sup>

<sup>1</sup>Centro i~mar, CeBiB, MASH, Universidad de Los Lagos, Camino a Chinquihue km6, Puerto Montt 580000, Chile.

<sup>2</sup>Centro i~mar, Universidad de Los Lagos, Camino a Chinquihue km6, Puerto Montt 580000, Chile.
 <sup>3</sup>Centro de Investigación en Ecosistemas de la Patagonia (CIEP), José de Moraleda 16, Coyhaique, Chile.
 <sup>4</sup>Center for Oceanographic Research COPAS Sur-Austral and COPAS COASTAL, Universidad de Concepción

pamela.fernández@ulagos.cl

The ability of seaweeds to adapt and/or acclimate to climate change can differ between different populations, depending on their local environmental history and phenotypic plasticity. Globally, many seaweed populations have been negatively affected by multiple anthropogenic factors such as ocean warming (OW). However, local drivers such as nitrogen (N) might modulate their physiological responses to OW. In this study, we compare the physiological responses and early development of *Macrocystis pyrifera* to different regimes of temperature (stable–fluctuating) from populations naturally exposed to fluctuating conditions of CO2, pH, temperature, and N. Fertile sporophylls of *M. pyrifera* were collected from populations with high (Las Docas and Punta Lavapiés) and less exposure to upwelling events (El Tabo and Punta de Parra). Temperature, pH and nutrient concentrations were constantly monitored in each locality. At the laboratory, *M. pyrifera* early stages (meiospore, gametes, and juvenile sporophytes) were exposed for three months to four temperature treatments: 16°C stable, 16°C fluctuating, 19°C stable and 19°C fluctuating, simulating the average temperatures during the spring-summer months and OW scenarios.

As a result, we observed great variability in the developmental (germination success, gametophytes growth, reproduction success) and physiological responses (growth and photosynthesis) among populations and temperature treatments, regardless of the upwelling effect. These differences might be given by the great variability registered in temperature and pH in each locality. However, the greatest differences among populations were found between gametophytes and juvenile sporophytes, suggesting different adaption capacities among multiple life stages of *M. pyrifera*.

# Resilience of a warm-temperate fucoid to a simulated marine heatwave: Exploring the interplay between life stages and tissues in relation to emersion

Sánchez de Pedro, R., Fernández, A.N., García-Sánchez, M.J., Flores-Moya, A. & Bañares-España, E.

Universidad de Málaga, Andalucía Tech, Departamento de Botánica y Fisiología Vegetal, Campus de Teatinos, 29010 Málaga, Spain \*rsdpc@uma.es

Marine heatwaves (MHW) represent one of the main climatic threats for seaweed assemblages, altering their functioning, structure, and ecosystem services. Experimental simulations allow to assess the potential impacts of MHWs, given the unpredictability of these events and frequently, the lack of responsive workflows to access the target population during these events.

This study explored the ability of the warm-temperate intertidal fucoid *Fucus guiryi* to respond to a simulated MHW. We aimed to evaluate the intraspecific response of different life stages (adult/juvenile/recruit) and blade types (vegetative / fertile–receptacles) in response to emersion, and to ascertain whether previous moderate MHWs in the area might impact its recruitment. The experimental design accounted for daily thermal and emersion fluctuations considering the most intense registered MHW event and maximum air temperatures in the study area.

Photophysiological evaluation revealed that receptacles were the most resilient tissue to the combined effects of heat and emersion, followed by canopy-protected embryos. Vegetative apices and plantlets exhibited marked declines in  $F_v/F_m$ , ETR<sub>max</sub> and NPQ<sub>max</sub> close to the MHW peak, due to the additive stressors, while embryos were more affected by the end of the experiment. During the recovery and end phases, recruit density dropped by 50% in the MHW treatment, being 80% of extruded structures unfertilized egg packets, while in the control treatment recruitment was successful and up to 35-times higher. *Fucus guiryi* was able to recover from the MHW, but more intense and frequent events might affect their reproductive output and compromise its long-term survival.

### Variable seasonal photosynthetic quotients of a kelp species – A mirror of the complex relationship between oxygen and carbon

Franke, K.<sup>1</sup>, Bartsch, I.<sup>2</sup>, Karsten, U.<sup>1</sup> & Graiff, A.<sup>1</sup>

<sup>1</sup>Institute of Biological Sciences, Applied Ecology and Phycology, University of Rostock, Germany <sup>2</sup>Alfred Wegener Institute, Helmholtz-Centre for Polar and Marine Research, Germany kiara.franke@uni-rostock.de

In the face of climate change, calculation of reliable net primary production rates (NPP) and carbon capture potential (CCP) of kelp forests has experienced increasing attention but estimations are still challenged by insufficient knowledge about the seasonal change of photosynthesis and carbon fixation. The kelp, *Laminaria hyperborea*, is one of the most important ecosystem engineers and primary producers in the coastal zone of the NE-Atlantic. We thus investigated the NPP and CCP of *L. hyperborea* at the island of Helgoland (North Sea, Germany) along a depth gradient (2, 4 and 6m) over one year. Under seasonal ambient (spring: 7°C, summer: 16°C, autumn: 14°C, winter: 6°C) and elevated temperatures (delta +4°C) at simulated seasonal in situ irradiances, we measured oxygen production (OP) and carbon fixation (CF) and thereof calculated seasonal photosynthetic quotients (PQs; n=6). Overall, temperature increase had a stimulating effect on CF, while OP was comparable between

TUESDAY 22<sup>nd</sup> August 2023 Room « Tali melen »

treatments within each season, revealing decreased PQs under elevated temperature treatments (PQs: ambient: 1.7-4.4, delta +4°C: 1.7-3.7). *L. hyperborea* exhibited a high photosynthetic plasticity along the depth gradient, but OP displayed a pronounced annual cycle that was not revealed by CF. OP was maximal during spring and summer, and decreased over the course of the year to approx. 50% of maximum values in winter. We will discuss the complex relationships between OP and CF and how resulting variable PQs may influence annual NPP estimations as well as CCP of kelp, in comparison to overestimated annual NPP based on one single PQ.

### The role of Lhcx isoforms in photoprotection mechanism in the diatom, *Thalassiosira pseudonana*

Nakayasu, M.<sup>1</sup>, Yoneda, K.<sup>2</sup>, Shimakawa, G.<sup>1</sup> & Matsuda, Y.<sup>1</sup>

<sup>1</sup>Marine Bioscience, School of Biological and Environmental Sciences, Kwansei Gakuin University, Japan <sup>2</sup>Faculty of Life and Environmental Sciences, University of Tsukuba, Japan hcr52781@kwansei.ac.jp

While low light conditions limit the energy utilizations by algae, light absorption exceeding the maximal capacity of photosynthesis enhances formation of toxic reactive oxygen species, which potentially causes photodamages. To prevent the high light-induced photodamage, photosynthetic organisms evolved numerous photoprotective strategies. Among these, energydependent fluorescence quenching (qE) provides a rapid mechanism to dissipate thermally the excessively absorbed energy. qE in diatoms is provided by a concerted action of Lhcx proteins and the multiple xanthophyll cycle pigments diatoxanthin and zeaxanthin. However, the exact mechanisms of the Lhcx activation is unknown.

In this study, we focused on the model centric diatom, *Thalassiosira pseudonana* that has 6 Lhcx proteins. To investigate the molecular mechanism of qE, we generated mutants defective of Lhcx1/2 or Lhcx6\_1. In the Lhcx1/2 knockout lines, qE stimulation under high light irradiations became slower compared to the wild type cells. This result indicate that Lhcx1/2 play an irreplaceable role in photoprotection. On the other hand, NPQ increased under high light irradiation in the Lhcx6\_1 knockout line. Nevertheless, Lhcx6\_1 protects PSII against photoinhibition.

### The violaxanthin cycle can provide Non-Photochemical Quenching in *Phaeodactylum tricornutum*

Giossi, C.E.<sup>1</sup>, Kroth, P.<sup>1</sup>, Lohr, M.<sup>2</sup> & Lepetit, B.<sup>1</sup>

<sup>1</sup>Plant Ecophysiology, Department of Biology, University of Konstanz, Konstanz, Germany <sup>2</sup>Institute of Molecular Physiology, Plant Biochemistry, Johannes Gutenberg University, Mainz, Germany chiara.giossi@uni-konstanz.de

Like all photosynthetic organisms, diatoms need efficient photoprotective strategies to avoid damage derived by excess light. One of these, the xanthophyll cycle, plays a key role in the onset and recovery of qE, the high-energy-state quenching component of Non-Photochemical Quenching (NPQ), a form of excess energy dissipation trough heat. Under high light, violaxanthin de-epoxidase (VDE) converts diadinoxanthin into diatoxanthin, generating qE; under low light, diatoxanthin is epoxidized back to diadinoxanthin thanks to zeaxanthin epoxidase (ZEP), restoring the cycle and thus tuning down NPQ. Diatoms display several paralogs of VDE and ZEP, which functions have not yet been completely elucidated. In this work, we characterised the physiology of different knockout mutants of the model diatom *Phaeodactylum tricornutum*. Under light stress, one of these mutants significantly accumulates

TUESDAY 22<sup>nd</sup> August 2023 Room « Tali melen »

and cycles pigments of the violaxanthin cycle, to the detriment of the classical diadinoxanthin cycle. However, their qE capacity is comparable to that of the *wild type*. With this work we show for the first time that both major xanthophyll cycles present in nature can provide NPQ in the same organism.

# Effect of heat stress on phototrophic biofilm photosynthesis in the context of climate change: a thermal dose approach

Laviale, M.<sup>1</sup>, Courcoul, C.<sup>2</sup>, Guyot, L.<sup>3</sup>, Baillard, V.<sup>1</sup>, Billoir, E.<sup>1</sup>, Boulêtreau, S.<sup>2</sup>, Leflaive, J.<sup>2</sup> & Jesus, B.<sup>3</sup>

<sup>1</sup>Université de Lorraine, CNRS, LIEC, F-57000 Metz, France <sup>2</sup>Laboratoire écologie fonctionnelle et environnement, Université de Toulouse, CNRS, INPT, UPS, Toulouse, France <sup>3</sup>Université de Nantes, Laboratoire Mer Molécules Santé, Nantes, France martin.laviale@univ-lorraine.fr

Climate change is threatening aquatic ecosystems worldwide, notably through the increase in heatwave intensity and frequency. Photosynthesis is one of the most heat-sensitive cellular processes, it is therefore necessary to better understand the heat stress effects on photosynthetic activity and corresponding primary productivity. Currently, these effects are not well documented due to the lack of a modeling approach able to integrate both temperature intensity and heat duration experienced during short extreme heat stress events. This is especially the case for phototrophic biofilms, for which the probability to face heat stress increases, in both fresh and marine ecosystems.

For this purpose, we implemented a laboratory controlled experimental set up (30 to 45°C, up to 3 h, using in vivo chlorophyll fluorescence as a proxy of photosynthetic activity) to expose to short-term heat stress benthic diatoms from shallow freshwaters or intertidal mudflats. The concept of "thermal dose" was then used to compute a dose of heat, defined as the product of time and an exponential function of temperature.

This approach allowed us to compare the tolerance and responsiveness to heat stress across a various set of experiments involving isolated algal strains, simplified synthetic communities or natural biofilms. We also showed that this modeling approach can be applied to in situ temperature data, for predicting the effects of heat stress events in situ. Our results show that even moderate heat stress can significantly impair primary productivity. While obtained on benthic diatoms, this approach offers promising research avenue for other (photosynthetic) organisms.

# Photoinactivation vs repair of photosystem II under thermal stress in epipelic and epipsammic microphytobenthos communities

Frankenbach, S., Bártolo, V. & Serôdio, J.

CESAM – Centre for Environmental and Marine Studies and Department of Biology, University of Aveiro, Campus de Santiago, 3810-193 Aveiro, Portugal s.frankenbach@ua.pt

Microphytobenthos (MPB) inhabiting intertidal flats are exposed to large and sudden changes in temperature during low tide. Often occurring simultaneously to exposure to direct sunlight, these extreme high or low temperatures are expected to negatively impact on photosynthesis and productivity by exacerbating the photoinhibitory effects of high light. This study addressed the photoinhibitory effects of short-term exposure to cold (5 °C) and moderate heat (35 °C) on MPB communities dominated

motile epipelic (EPL) and immotile epipsammic (EPM) diatom species, by evaluating the seasonal variation of photoinactivation and repair of photosystem II (PSII). Samples were collected from two sedimentary intertidal sites of the Ria de Aveiro (Portugal) at four occasions along one year.

The susceptibility to PSII photoinactivation and the counteracting repair capacity were measured by the constant rates kPI and kREC, respectively, by measuring the relative decrease in the maximum quantum yield of PSII caused by exposure to high light (%Fv/Fm) in untreated and lincomycin-treated samples. Overall, the exposure to extreme low and high temperatures was found to affect more the PSII repair capacity than the PSII photoinactivation, causing large decreases in kREC but minor increases in kPI. These effects varied seasonally, possibly indicating and a role of thermoacclimation: exposure to moderate heat had stronger effects in cold-acclimated samples (winter) while exposure to cold resulted in stronger effects in heat-acclimated samples (summer). Although the general pattern was similar for the two types of MPB communities, EPM samples tended to show larger seasonal variations than EPL ones.

# Motility of epipelic diatoms: inter- and intra-specific variability of its photoprotective role under a realistic light microgradient

Morelle, J., Bastos, A, Frankenbach, S., Frommlet, J. & Serôdio, J.

CESAM – Centre for Environmental and Marine Studies and Department of Biology, University of Aveiro, Campus de Santiago, 3810-193 Aveiro, Portugal jerome.morelle@ua.pt

Epipelic benthic diatoms are capable of directed motility, which has been hypothesized to be a major factor explaining their evolutionary success, diversification, and productivity. A long-standing idea is that they use motility as a form of photoregulation, to adjust their position in the photic zone, optimizing photosynthesis and avoiding excessive photoinhibitory irradiances. The fact that the sedimentary photic zone is very thin (1-3 mm) might be key to such a strategy, as cells can traverse these distances within a short time (< 30 min). However, due to methodological limitations in reproducing such light microgradients for experimentation, the degree to which diatom motility alleviates photoinhibition remained untested.

Here we present a new experimental setup that allows us to reproduce light gradients representative of those experienced by benthic diatoms living in exposed intertidal zones. By combining microfluidics and cellular bioimaging of chlorophyll fluorescence, we were able to track movement and photophysiology of various epipelic diatoms species in response to light microgradients.

The results show that motility can indeed be used to select optimal light levels and limit photodamage to the photosynthetic apparatus. However, the use of this form of photoregulation does not seem to be favored equally among different species, nor by all cells of the same population. This result highlights the inter- and intra-species variability regarding the ability to endure high light doses. Given the potential cost of motility, this raises the question of when motility is favored over other forms of photoprotection.

# Using *Cyclotella cryptica* as a new model to understand the physiological, molecular and genetic regulation of trophic mode switching in facultative heterotrophic diatoms

Jensen, E.L.<sup>1</sup>, Aboudagga, C.<sup>1</sup>, Maes, A.<sup>2</sup>, Bailleul, B.<sup>1</sup>, Choquet, Y.<sup>1</sup> & Falciatore, A.<sup>1</sup>

<sup>1</sup>Laboratoire de Biologie du chloroplaste et perception de la lumière chez les micro-algues, UMR7141, CNRS, Sorbonne, Institut de Biologie Physico-Chimique, Paris, 75005, France <sup>2</sup>Laboratoire de Biologie Moléculaire et Cellulaire des Eucaryotes, UMR8226, CNRS, Sorbonne, Institut de Biologie Physico-Chimique, Paris, 75005, France jensen@ibpc.fr

Diatoms are among the most abundant photosynthetic eukaryotes in oceans, responsible of about 40 percent of the total marine primary production. Over millions of years of evolution, diatoms have adapted to inhabit a wide diversity of ecological niches, developing a high resilience and tolerance to varying salt concentrations, temperature and light availability. While a few species (*e.g.* from the *Nitszchia* genus) have lost photosynthesis, the vast majority of diatom species are described as obligate phototrophs. However, facultative heterotrophic growth has been observed in several diatom genera and some aspects of their nutrition characterized, but the physiological and molecular cues that underpin the switching between heterotrophic, phototrophic and mixotrophic metabolisms have been largely overlooked.

The centric diatom *Cyclotella cryptica* was chosen for this study because of its ability to both grow using only photosynthesis as energy source or in complete darkness using glucose as external source of reduced carbon. We characterized its growth, photosynthesis and respiration in phototrophic, heterotrophic and mixotrophic conditions, and then contrasted them to transcriptomic data. Our findings reveal a very complex genetic and metabolic regulation of *C. cryptica* in all trophic modes, and evidenced glucose as a major drive of these responses, as its effects seem stronger than those triggered by light. In parallel, taking advantage of its heterotrophic growth, we are developing genetic tools in *C. cryptica* for its future use as a model diatom to perform loss-of-function studies of key photosynthetic and plastid functions.

# Abrupt and acclimation responses to changing temperature elicit divergent physiological effects in the diatom *Phaeodactylum tricornutum*

Rehder, L., Rost, B. & Rokitta, S.

Alfred-Wegener-Institute Bremerhaven, Germany linda.rehder@awi.de

Temperature as a universal driver affects all organisms. Marine phytoplankton from temperate and polar regions already undergo natural temperature variability on time scales of seasons, mixing and tidal events. Additionally, rising mean sea surface temperatures as well as heatwave events are projected to increase in frequency and thereby strongly affect phytoplankton growth and biomass traits. We hypothesized that resulting phenotypes originate from deviating temperature sensitivities of underlying physiological processes. We used membrane-inlet mass spectrometry to assess photosynthetic and respiratory  $O_2$  and  $CO_2$  fluxes in response to abrupt temperature changes as well as after acclimation periods (6°C vs. 15°C) in the model diatom *Phaeodactylum tricornutum*. Abrupt temperature changes

caused immediate over- or undershoots in most physiological processes, i.e. photosynthetic oxygen release, photosynthetic carbon uptake and respiratory oxygen release. Over acclimation timescales, cells were, however, able to re-adjust their physiology and revert to phenotypic 'sweet spots'. Interestingly, respiratory  $CO_2$  release was generally inhibited under high temperature and stimulated under low temperature settings, on abrupt as well as acclimation time scales. Such behavior was likely caused by an alternative electron export from the chloroplasts into the mitochondria to stabilize plastidial ATP:NADPH ratios and thus maximize photosynthetic carbon assimilation. Currently, we are looking into the thermal performance of Arctic key species in multiple functional traits and their underlying physiological processes to determine metabolic bottlenecks under future temperature scenarios to improve our understanding about detrimental temperature effects.

### A multi-level approach to investigate the dynamics and spatial heterogeneity of $N_2$ fixation and photosynthesis in *Trichodesmium*

Hania, A.<sup>1,2</sup>, López-Adams, R.<sup>1</sup>, Míšková, A.<sup>1,2</sup>, Piwosz, K.<sup>3</sup>, Prášil, O.<sup>1,2</sup> & Eichner, M.<sup>1</sup>

<sup>1</sup>Laboratory of Photosynthesis, Institute of Microbiology of the Czech Academy of Sciences, Centre Algatech, Třeboň, Czech Republic <sup>2</sup>Egoulta of Science, University of Scuth Bohemin, Česká Budžiovice, Czech Borwhlie

<sup>2</sup>Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic

<sup>3</sup>Department of Fisheries Oceanography and Marine Ecology, National Marine Fisheries Research Institute, Gdynia, Poland

hania@alga.cz

The filamentous cyanobacterial genus *Trichodesmium* is globally one of the most abundant  $N_2$ fixers. Since nitrogenase, the enzyme converting  $N_2$  to  $NH_4^+$ , is irreversibly inhibited upon  $O_2$ exposure, *Trichodesmium* has evolved a special combination of time- and space-separation strategies to coordinate  $N_2$  fixation and  $O_2$  production. Photosynthesis was shown to be downregulated during the peak of  $N_2$  fixation, photosynthetic activity states were reported to switch on time scales of minutes, while nitrogenase was suggested to be confined into diazocytes. However, the overall mechanism of how *Trichodesmium* reconciles these two mutually exclusive processes remains unresolved, possibly due to the multitude of different protocols and/or strains employed among studies.

Herein, we compared two laboratory strains of *T. erythraeum*, IMS101 and NIBB1067, and investigated the dynamics and spatial heterogeneity of  $N_2$  fixation and photosynthesis. We used a multi-level approach employing techniques such as mRNA Catalyzed Reported Deposition Fluorescence *in situ* Hybridization (mRNA CARD-FISH) to localize *nifH* and *psbA* expressions, Nanoscale Secondary Ion Mass Spectrometry (NanoSIMS) to infer  $N_2$  and CO<sub>2</sub> fixation, and Fluorescence Lifetime Imaging Microscopy (FLIM) to image photosynthetic pigments. Overall, our results displayed larger heterogeneity in IMS101 than in NIBB1067. The absence of spatial heterogeneity in the latter suggests that a temporal separation strategy might be more relevant and that other O<sub>2</sub>-lowering mechanisms are involved in the protection of nitrogenase. While these strain-specific differences advance our understanding of  $N_2$  fixation *vs* photosynthesis separation in *Trichodesmium*, further investigation is needed to test whether our laboratory results are also applicable to natural *Trichodesmium* colonies.

#### Titrating nonphotochemical quenching to develop a comprehensive model of photosynthesis regulation in the diatom *Phaeodactylum tricornutum*

Croteau, D.<sup>1</sup>, Jaubert, M.<sup>1</sup>, Bouly, J.-P.<sup>2</sup>, Falciatore, A.<sup>1</sup> & Bailleul, B.<sup>1</sup>

<sup>1</sup>CNRS, Chloroplast Biology and Light-sensing in Microalgae-UMR7141, France <sup>2</sup>Institut des Sciences du Calcul et des Données, Sorbonne Université, Paris, France croteau@ibpc.fr

To thrive, photosynthetic organisms must maximize the harvest of light energy driving carbon fixation while protecting themselves from excess light which can cause oxidative damage to photosystem II (PSII) and cause photoinhibition. Diatoms are known as specialists of turbulent light regime and therefore represent an ideal group to study the dynamic light responses of photosynthesis. Among them, the nonphotochemical quenching (NPQ) releases excess light energy as heat and is induced when the linear electron flow is saturated. In diatoms, the amplitude of NPQ strictly depends on the abundance of two molecular actors, the xanthophyll pigment diatoxanthin and Lhcx proteins. We modulated NPQ in 14 mutant strains of the model diatom Phaeodactylum tricornutum expressing different amounts of Lhcx1. This "molecular titration" provides us with a powerful experimental system: the amplitude of NPQ can be tuned, all other things being equal, to disentangle different phenomena involved in the functional light-response of photosynthesis. We show that under light stress, photoinhibition is counteracted by the synergistic action of NPQ and the enzymatic repair cycle of PSII. By downregulating the efficiency of PSII, NPQ can also stimulate cyclic electron flow around PSI in brightto-dim light transients, which increases the ATP/NADPH ratio. These results suggest that the NPQ is a good entry point to develop a phenomenological model of photosynthesis regulation in diatoms. Our latest results also suggest NPQ amplitude largely influences the patterns of genes expression to cope with high light shift.

#### Identifying the proteins underpinning the diatom pyrenoid

Nam, O. & Mackinder, L.

Centre for Novel Agricultural Products (CNAP), Department of Biology, University of York, York YO10 5DD, United Kingdom \*onyou.nam@york.ac.uk

Diatoms are a major phytoplankton group responsible for up to 20% of global CO<sub>2</sub> fixation. They efficiently fix carbon from the aqueous environment by running a CO<sub>2</sub>-concentrating mechanism (CCM) to concentrate CO<sub>2</sub> at the active site of Rubisco packaged in a subcellular compartment called the pyrenoid. Despite the importance of diatom CO<sub>2</sub>-fixation, the pyrenoid protein composition is still not well known. Here, we aim to identify the proteins forming the diatom pyrenoid using *Thalassiosira pseudonana*. Recently, we established a high-throughput fluorescent protein (FP) tagging pipeline using modular cloning (MoClo) framework based on the Golden Gate system for episomal delivery via conjugation. The potential pyrenoid proteins were identified by affinity purification coupled with mass spectrometry (AP-MS), using Rubisco, the major pyrenoid component, as bait. Subsequently, the identified proteins were fluorescently tagged, and their spatial distribution was analyzed by confocal microscopy.

To identify additional pyrenoid proteins and to build a pyrenoid protein-protein interactome, we performed AP-MS utilizing the FP-tagged lines as baits. This study reports several uncharacterized

proteins localized to distinct sub-regions of the *T. pseudonana* pyrenoid providing novel insights into diatom pyrenoid structure and function.

# The influence of light spectra, intensity and photosynthesis on the motility behaviour and photoacclimation of two species of benthic diatoms

Underwood, G.J.C.<sup>1,2</sup>, Slee, N.J.D.<sup>1</sup>, Yamada, N.<sup>3</sup>, Kroth, P.G.<sup>3</sup> & Lepetit B.<sup>3</sup>

<sup>1</sup>School of Life Sciences, University of Essex, U.K.
<sup>2</sup>Zukunftskolleg, University of Konstanz, Germany
<sup>3</sup>Department of Biology, University of Konstanz, Germany gjcu@essex.ac.uk

Many species of pennate diatoms are motile, and motility is a key adaptation for microphytobenthic diatoms that live in marine, estuarine and freshwater sedimentary environments. Motility allows cells move to the surface of sediment to photosynthesise after physical disturbance, and in many intertidal species is linked to endogenous migratory rhythms aligned with day to night and tidal cycles. Motility also allows cells to reposition themselves into optimal conditions in the steep and varying light gradients within the sediment matrix. Motile diatoms respond to light through a combination of positive and negative phototaxis (directional movement) and photokinesis (changing speed), and these responses differ between species and with the colour of light. It is known that diatoms possess genes that code for hytochromes, cryptochromes and aureochromes that respond to red/far red and blue light. Some research indicates light sensing takes place at the apices of cells, other research indicates a role for the chloroplast. Experimental manipulations of cultures of two benthic diatom species, Nitzschia captiva and Cylindrotheca closterium, found differing positive and negative phototactic behaviours in response to varying intensities of light (comparing both irradiance, µmol m<sup>-2</sup> s<sup>-1</sup>, and photosynthetically absorbed radiation, Qphar) across a range of light spectral conditions (ranging from blue to far-red light). Each species showed changes in their motility behaviour when photosynthesis was inhibited (using the inhibitor DCMU). The two diatom species exhibited a range of differential photophysiological acclimations associated with this set of conditions and behaviours, suggesting some role for chloroplast signalling in regulating motility. The potential controls, and importance of these responses in enabling diatoms to thrive in highly variable environmental conditions is discussed.

# Dynamic and sustained non-photochemical quenching under light/dark illumination and nutrient starvation in phytoplankton in culture and in natural communities

#### Lacour, T.

*Ifremer, PHYTOX, Laboratoire PHYSALG, F-44000 Nantes, France* thomas.lacour@ifremer.fr

Xanthophyll cycle (XC) related non-photochemical quenching (NPQ), which is present in most photoautotrophs, allows dissipating excess light energy. Generally, it can be reversed for photosynthesis to resume when light intensity is brought back to more optimal intensity. The presence of diatoxanthin (Dt) or zeaxanthin (Z) at steady state in photoautotrophs acclimated to moderate irradiance suggests that they use XC related NPQ to dissipate light energy absorbed in excess under constant light. We investigated the characteristics of NPQ in unialagal cultures of the haptophyte *Tisochrysis lutea*, the

#### TUESDAY 22<sup>nd</sup> August 2023 Room « Tali melen »

dinophyte *Alexandrium minutum* and in the diatom *Chaetoceros mulleri* under L/D cycles and N starvation and in natural communities from the Bay of Biscay in May 2021 and 2022. All species in culture as well as natural communities showed strong sustained NPQ (NPQs), particularly under N starvation, which was impossible to quantify using PAM fluorometry. The potential role of NPQs in the photophysiological plasticity of microalgae, especially under adverse growth conditions, is discussed.

### PLENARY

#### Light in the life of marine diatoms

#### Falciatore, A.

Laboratory of Chloroplast Biology and Light-sensing in Microalgae, Institut de Biologie Physico-Chimique Paris, France angela.falciatore@ibpc.fr

Diatoms are a major class of phytoplankton, standing at the crossroads of several evolutionary lineages. They represent the most species-rich class of microalgae and are distributed worldwide, from tropical and subtropical regions to polar ecosystems. Thus, the diversity of diatom lifestyles and survival strategies can likely be attributed to their exceptional ability to adapt to diverse environments.

Light is the primary source of energy for photosynthetic organisms but also a key source of information on the surrounding environment. It is well established that diatoms have developed highly effective systems for optimizing light harvesting and energy generation from photosynthesis. Yet, little is known on the role of light sensing in the acclimation mechanisms, which synergistically control diatom cell growth and distribution within the ecological oceanic provinces they inhabit. To address these questions, we are performing integrated analyses of diverse but highly interconnected light-driven processes. By developing genomic and genetic resources for *Phaeodactylum tricornutum*, currently the most established experimental model for diatom molecular research, and by integrating genome-enabled and (eco)physiology approaches, we started to unveil new molecular players of diatom photoregulation. They include key regulators of photosynthesis and plastid photoprotection, photoreceptor variants, and a long-sought circadian clock controlling diatom responses to periodic light:dark cycles. Comparative functional investigations in diverse species and the analysis of the distribution of light regulators in the marine environment support hypothesis that light-driven processes are key players of diatom persistence. Providing new perspectives on how photoregulators evolved, diversified and act in aquatic environments strongly structured by light, these studies also highlight that integration of laboratory and environmental studies are both timely and essential to understand the life of algae in complex ecosystems.

#### SYMPOSIUM 03 "Phylogenetics, Phylogenomics and Evolution"

#### **KEYNOTE**

## Phylotranscriptomics unveil a Paleoproterozoic-Mesoproterozoic origin and deep relationships of the Viridiplantae

Yang, Z.<sup>1</sup>, Xiao, S.<sup>2</sup>, De Clerck, O.<sup>3</sup>, Leliaert, F.<sup>4</sup>, Zhong, B.<sup>1</sup>

<sup>1</sup>College of Life Sciences, Nanjing Normal University, Nanjing, China.
 <sup>2</sup>Department of Geosciences and Global Change Center, Virginia Tech, Blacksburg, VA, USA.
 <sup>3</sup>Phycology Research Group and Center for Molecular Phylogenetics and Evolution, Ghent University, Ghent, Belgium.
 <sup>4</sup>Meise Botanic Garden, Meise, Belgium.
 bjzhong@gmail.com.

The Viridiplantae (green plants) comprise two main clades, the Chlorophyta (which includes a large diversity of marine and freshwater green algae) and the Streptophyta (which consists of the freshwater Charophyta and the land plants). The early-diverging lineages of the Chlorophyta, informally referred to as the prasinophytes, are a paraphyletic assemblage of mainly planktonic green algae. One of these lineages, Prasinodermophyta, has recently been identified as the earliest diverging lineage of the Viridiplantae based on phylogenomic evidence. Resolving the early evolutionary relationships of the green plants is important for understanding the impact of ancient green algal diversity on marine ecology and geochemistry, but has been proven difficult given the ancient nature of the diversification events. By performing large-scale phylogenomic analyses through extensive taxon and gene sampling, we resolve deep relationships and confirm the Prasinodermophyta as the earliest-diverging lineage of green plants. We further unveil that incomplete lineage sorting (ILS) was the main cause of discordance concerning the position of the Prasinodermophyta. Molecular dating analyses with four calibration strategies suggest that crown-group green plants and crown-group Prasinodermophyta evolved in the Paleoproterozoic-Mesoproterozoic and the origin of the Viridiplantae.

### ORALS

# Phylogenomics reveals the evolutionary origin of lichenization in chlorophyte algae

Puginier, C.<sup>1</sup>, Libourel, C.<sup>1</sup>, Otte, J.<sup>2</sup>, Skaloud, P.<sup>3</sup>, Haon, M.<sup>4,5</sup>, Grisel, S.<sup>4,5</sup>, Petersen, M.<sup>6</sup>, Berrin, J-G.<sup>4,5</sup>, Delaux, P-M.<sup>1</sup>, Dal Grande, F.<sup>2,7,8</sup> & Keller, J.<sup>1</sup>

<sup>1</sup>Laboratoire de Recherche en Sciences Végétales (LRSV), Université de Toulouse, CNRS, UPS, INP Toulouse, 31320 Castanet-Tolosan, France.

<sup>2</sup>Senckenberg Biodiversity and Climate Research Centre (SBiK-F), Senckenberganlage 25, Frankfurt am Main, Germany

#### THURSDAY 23<sup>rd</sup> August 2023 Room « Tali ebrel »

<sup>3</sup>Department of Botany, Faculty of Science, Charles University, Benátská 2, CZ-12800 Praha 2, Czech Republic <sup>4</sup>INRAE, Aix Marseille Université, UMR1163 Biodiversité et Biotechnologie Fongiques (BBF), 13009 Marseille, France

<sup>5</sup>INRAE, Aix Marseille Université, 3PE Platform, 13009 Marseille, France

<sup>6</sup>High Performance Computing & Analytics Lab, University of Bonn, Friedrich-HirzebruchAllee 8, 53115 Bonn, Germany

<sup>7</sup>LOEWE Centre for Translational Biodiversity Genomics (TBG), Senckenberganlage 25, Frankfurt am Main, Germany

<sup>8</sup>Department of Biology, University of Padova, Italy

camille.puginier@univ-tlse3.fr

Chlorophytes algae display an astonishing diversity of habitats and can be found in marine, freshwater and terrestrial environments. Among the latter, around 120 species, can associate with lichen-forming fungi (LFF) to form mutualistic symbioses known as lichens.

The evolutionary history of lichenization in Chlorophytes remains elusive. New approaches, such as comparative phylogenomics, have previously helped deciphering evolutionary histories of other symbioses but they couldn't be performed for lichenization due to the lack of genomes in this clade. Lichen evolution has been suggested to result from a convergent evolution. However the molecular mechanism regulating the interaction and their evolutionary dynamics remains elusive. To assess these questions, we sequenced 12 genomes and transcriptomes of LFA and nLFA (non-lichen-forming algae) and combined them with 129 other available genomes and transcriptomes. Using ancestral state reconstruction and comparative phylogenomics approaches, we showed that lichenization was gained multiple times independently within the Chlorophytes. Candidate genes and functional families linked to lichenization were identified. Among them, the glycoside hydrolase 8 (GH8) is only present in Trebouxiophyceae and expanded in LFA. We also demonstrated that this GH8 originated from a horizontal gene transfer (HGT) and is responsible for the degradation of the lichenan, a carbohydrate present in LFF. Then, GH8 could be involved in the LFA accommodation within the lichen thallus. Overall, we showed that (1) lichenization is a convergence evolution in Chlorophytes and that (2) gene family expansions and HGT might have provided the bases for lichenization to evolve.

### Genome-wide comparisons reveal extensive divergence within the common lichen photobiont genus, *Trebouxia*

Poquita-Du, R.C.<sup>1,2</sup>, Otte, J.<sup>1</sup>, Calchera, A.<sup>1,2</sup> & Schmitt, I.<sup>1,2,3</sup>

<sup>1</sup>Molecular Evolutionary Biology Group, Senckenberg Biodiversity and Climate Research Center, Georg-Voigt-Straße 14, Frankfurt am Main, Germany, 60325

<sup>2</sup>LOEWE Center for Translational Biodiversity Genomics, Senckenberganlage 25, Frankfurt am Main, Germany, 60325

<sup>3</sup>Institute of Ecology, Diversity and Evolution, Max-von-Laue-Str. 13, Frankfurt am Main, Germany 60438 rosacelia.du@senckenberg.de

The green algal genus, *Trebouxia*, is a keystone photobiont of many lichen-forming fungi. There are four recognized clades within *Trebouxia* (A, C, I, S), which are largely distinguished by differences in their pyrenoid structures. While the different clades are known to display a wide range of environmental tolerances, it is unknown how these differences are reflected in their genomes. Here, we generated PacBio-based *de novo* genome assemblies from six *Trebouxia* strains, isolated from *Umbilicaria phaea* (*Trebouxia* clade A) and *U. pustulata* (*Trebouxia* clade S) found at low and high elevations in California

and Europe. Specifically, we aim to examine genome sequence divergence among these *Trebouxia* strains and identify gene groups that may contribute to niche adaptation.

*De novo* assemblies of the six *Trebouxia* strains yielded highly-complete genomes (~96% average BUSCO completeness based on Chlorophyta), with assembly sizes ranging from 63.08-74.14 Mb and contained 14,109-18,870 genes. The number of proteins vary across different strains, ranging from 16,367-21,441, of which 25-37% are unique for each strain. In particular, we found differences in genes coding for proteins associated to photosynthesis, among and even within *Trebouxia* clades, suggesting differences in physiological capacities related to photosynthetic performance. Functional analysis also showed protein families which are shared and distinct between clades. Overall, we show that there is substantial genomic divergence within the algal genus *Trebouxia* which likely contributes to the genus' large ecological amplitude.

### The chloroplast genome of early diverging Cladophorales represents the missing link to hairpin chromosomes

Bringloe, T.T.<sup>1</sup>, Uthanumallian, K.<sup>1</sup>, Bjornson, S.<sup>1</sup>, Leliaert, F.<sup>2</sup>, Wetherbee, R.<sup>1</sup> & Verbruggen, H.<sup>1</sup>

<sup>1</sup>University of Melbourne, Australia <sup>2</sup>Meise Botanic Garden, Belgium heroen.verbruggen@gmail.com

The chloroplast genomes of Cladophorales (Ulvophyceae) are unique among green algae in that they are fragmented into single stranded chromosomes that fold into hairpin structures. The sequence of events leading away from conventional chloroplast architecture to highly reduced linear molecules has remained a mystery. Here, we sequenced and assembled a chloroplast genome belonging to a new species of *Okellya*, a member of an early diverging lineage within the Cladophorales. Using a combination of long and short read shotgun sequencing, we were able to confirm the chloroplast genome as circular, but with highly reduced size and gene content compared to conventional green algal genomes. The 29,168 bp genome houses 20 core chloroplast genes, the 16S RNA, 8 tRNAs and an ORF of unknown function. An approx. 4,500 bp segment of DNA with repeat elements separates the otherwise condensed genome. Our investigations pinpoint gene losses and the origin of the aberrant genetic code in Cladophorales plastid genomes, provide new insights into nuclear-encoded plastid genome structure.

# Algal metagenomics provides insights into land plant terrestrialisation

Bowles, A.M.C.<sup>1,2</sup>, Williams, T.A.<sup>2</sup>, Donoghue, P.J.C.<sup>2</sup> & Williamson, C.J.<sup>1</sup>

<sup>1</sup>*MicroLab*@*Bristol Research Group, Bristol Glaciology Centre, School of Geographical Sciences, University of Bristol, UK.* 

<sup>2</sup>Bristol Palaeobiology Group, School of Biological Sciences and School of Earth Sciences, Life Sciences Building, University of Bristol, UK.

zl20161@bristol.ac.uk

The diversification of the first land plants from their algal ancestors, around 650 million years ago, transformed the biosphere. However, the geological and biological conditions of this major transition

#### THURSDAY 23<sup>rd</sup> August 2023 Room « Tali ebrel »

are poorly understood. This period in Earth history falls into the Cryogenian, in which the Earth was almost completely covered in ice, similar to modern glacial environments. Today, Zygnematophycean glacier algae inhabit aquatic surface ice environments, tolerating a host of terrestrial stressors (e.g. extremes of temperature, UV radiation and water availability). Here, by studying their metagenomes and physiological adaptations, we investigate whether the adaptations of extant glacier algae represent ancient exaptations important for the transition of ancestral plants from water onto land.

Phylogenetic analysis confirms the placement of glacier algae in the sister lineage to land plants as well as exploring their diversification within the Zygnematophyceae. Comparative genomic analysis identifies large-scale patterns of gene novelty and expansion in the ancestor of land plants and Zygnematophyceae as well as the ancestor of land plants. Functional annotation demonstrates that genes novel to land plants and Zygnematophyceae were associated with phytohormone signalling and stress responses, whilst expanded gene families in the ancestor of land plants were linked to stomatal and cell wall development. Further comparative genomics identifies gene family loss and contraction in the ancestor of Zygnematophyceae, suggesting that secondary simplification is masking the complexity of the ancestor of Zygnematophyceae and land plants. This analysis provides key insight into algal exaptations crucial for land plant terrestrialisation.

#### Cryptic diversification in a widespread phytoplankton at low latitudes

Bendif, E.M.<sup>1,2,3</sup>, Archontikis, O.<sup>2</sup>, Schiffrine, N.<sup>1</sup>, Probert, I.<sup>4</sup>, Young, J.<sup>5</sup>, Rickaby, R.E.M.<sup>2</sup> & Filatov, D.<sup>3</sup>

<sup>1</sup>Institut des sciences de la mer de Rimouski (ISMER), Université du Québec à Rimouski, Rimouski, Canada <sup>2</sup>Department of Earth Sciences, University of Oxford, Oxford, UK <sup>3</sup>Department of Plant Sciences, University of Oxford, Oxford, UK <sup>4</sup>Sorbonne Université – CNRS, Roscoff Culture Collection, FR2424 Station Biologique de Roscoff, Roscoff, France <sup>5</sup>Department of Earth Sciences, University, College London, London, UK

<sup>5</sup>Department of Earth Sciences, University College London, London, UK elmahdi\_bendif@uqar.ca

Phytoplankton account for nearly half of global primary productivity and strongly affect the global carbon cycle, yet little is known about the forces that drive the evolution of these keystone microscopic organisms. Here we combine morphometric data from extant strains of the tropical coccolithophore *Gephyrocapsa oceanica* with genomic analyses to assess the genetic processes underlying its evolution. We demonstrate that modern diversity in *G. oceanica* originated in a rapid species radiation during the last interglacial (130-110ka), as we retrieved 3 genetic lineages with similar morphologies. Further environmental analyses suggest that sea surface temperature variability relate to these lineages, suggestive of a potential ecological selection. We then compared the two important *Gephyrocapsa* complex (*G. huxleyi* vs *G. oceanica*) to assess the adaptive process underlying their diversification. Overall, our study sheds light on the complex microevolution of *G. oceanica* and highlights the importance of considering microdiversity within phytoplankton species when studying their ecological and biogeochemical roles in the global ecosystem.

# Shedding light on the noncoding transcriptome of the ocean plankton

Debit, A.<sup>1‡</sup>, Charton, F.<sup>1‡</sup>, Pierre-Elies, P.<sup>1</sup>, Bowler, C.<sup>1</sup> & Cruz de Carvalho, H.<sup>1,2‡</sup>

<sup>‡</sup>Equal contribution

#### THURSDAY 23<sup>rd</sup> August 2023 Room « Tali ebrel »

<sup>1</sup>Institut de Biologie de l'ENS (IBENS), Département de Biologie, École normale supérieure, CNRS, INSERM, Université PSL, 75005 Paris, France <sup>2</sup>Université Paris Est-Créteil (UPEC), Faculté des Sciences et Technologie, 61, avenue du Général de Gaulle 94000 Créteil, France cruz@biologie.ens.fr

Long time considered as transcriptional noise, long non protein coding RNAs (lncRNAs) are gaining momentum as key regulators of cellular processes in eukaryotic cells. Presenting very little sequence conservation, these transcripts show nonetheless cross-kingdom conserved functions and highly specific expression regulation, namely in response to environmental cues and development. We have previously shown the occurrence of lncRNAs in diatoms, namely in *Phaeodactylum tricornutum*, many of which being expressed under specific stress conditions such as phosphate, nitrogen and hyposalinity. Recently, we have also shown that in this pleiomorphic diatom, lncRNA expression provides a cell-type specific signature suggesting that lncRNAs regulating cell identity preceded the evolution of multicellularity.

In order to expand lncRNA detection in a non-species-specific way, we developed a majority-voting pipeline to identify lncRNAs using a metaTool approach. This metaTool combines the use of 10 different tools and uses a majority voting approach. Based on the principle that using multiple and diverse tools will lead to a single reliable choice, we have generated an extensive catalogue of lncRNAs from transcriptomic libraries of around 400 marine protist species originating from The Marine Microbial Eukaryote Transcriptome Sequencing Project (MMETSP), covering the main branches of the tree of life. I will present an overview of the data obtained which reveals for the first time the large-scale expression of lncRNAs in the eukaryotic plankton of the sunlit ocean.

#### **KEYNOTE**

# Towards a comprehensive phylogeny and classification of the Ochrophyta

#### Eliáš, M.

Department of Biology and Ecology, Faculty of Science, University of Ostrava, Ostrava, Czech Republic marek.elias@osu.cz

Ochrophyta, also known as Heterokontophyta or stramenopile algae, constitute the most specious group of algae (provided that land plants are not considered to be just weird green algae) with an impresive range of morphologies from solitary flagellates to highly complex large multicellular kelps. Exploring the ochrophyte diversity and phylogeny has been a steady process driven both by curiosity of individual researchers and methodological progress of systematic biology in general, including the advent of molecular phylogenetics that was extremely instrumental for revising previous concepts of phylogeentic relationships among ochrophytes and delimitation of major lineages. However, only with the advent of phylogenomics, exploiting sequence data from nuclear as well as organellar genomes, the picture of the backbone of the ochrophyte phylogeny has been settling down, although important unresolved questions or incongruences among different data sets remain to be corted out. In my talk I will summarize and discuss published as well as unpublished results of the recent work in my research group or in collaboration with other colleagues that substantially advance our understandning of the major outlines of the ochrophyte phylogenetic tree. Topics presented will include recongnition of a novel ochrophyte class, identification of a putative ochrophyte lineage that has secondarily lost the plastid, expansion of the class Eustigmatophyceae by exploring environmental DNA data, and the progress in resolving the

deepest brachhes of the ochrophyte phylogeny. I will also provide some perspectives for future work towards a truly comprehensive phylogeny and classification of the Ochrophyta.

#### ORALS

# Phylotranscriptomics reveals the reticulate evolutionary history of a widespread diatom species complex

Ciftci, O.<sup>1,2,9,11</sup>, Alverson, A.J.<sup>3</sup>, van Bodegom, P.<sup>2</sup>, Roberts, W.R.<sup>3</sup>, Mertens, A.<sup>4</sup>, van de Vijver, B.<sup>5,6</sup>, Trobajo, R.<sup>7</sup>, Mann, D.G.<sup>7,8</sup>, Pirovano, W.<sup>9</sup>, van Eijk, I.<sup>10</sup> & Gravendeel, B.<sup>11,12</sup>

<sup>1</sup>Interface Geochemistry, GFZ German Research Centre for Geosciences, Potsdam, Germany
<sup>2</sup>Institute of Environmental Sciences, Leiden University, Leiden, The Netherlands
<sup>3</sup>Department of Biological Sciences, University of Arkansas, Fayetteville, Arkansas, USA
<sup>4</sup>Diatomella, Overasselt, The Netherlands
<sup>5</sup>Meise Botanic Garden Meise, Meise, Belgium
<sup>6</sup>University of Antwerp, Department of Biology–ECOBE, Wilrijk, Belgium
<sup>7</sup>IRTA-Institute for Food and Agricultural Research and Technology, Catalonia, Spain
<sup>8</sup>Royal Botanic Garden Edinburgh, Scotland, UK
<sup>9</sup>BaseClear B.V, Leiden, the Netherlands
<sup>10</sup>Bayer Crop Science, The Netherlands
<sup>11</sup>Naturalis Biodiversity Center, Leiden, The Netherlands
<sup>12</sup>Radboud Institute for Biological and Environmental Sciences, Nijmegen, The Netherlands
ozan.ciftci@gfz-potsdam.de

In contrast to surveys based on a few genes that often provide limited taxonomic resolution, transcriptomes provide a wealth of genomic loci that can resolve relationships among taxonomically challenging lineages. Diatoms are a diverse group of aquatic microalgae that includes important bioindicator species and many such lineages. One example is Nitzschia palea, a widespread species complex with several morphologically defined taxonomic varieties, some of which are critical pollution indicators. Morphological differences among the varieties are subtle and phylogenetic studies based on a few genes fail to resolve their evolutionary relationships. We conducted morphometric and transcriptome analyses of ten Nitzschia palea strains to resolve the relationships among strains and taxonomic varieties. Nitzschia palea was resolved into three clades, one of which corresponds to a group of strains with narrow linear-lanceolate valves. The other morphological group recovered in the shape outline analysis was not monophyletic and consisted of two clades. Gene-tree concordance analyses and phylogenetic network estimations revealed patterns of incomplete lineage sorting and gene flow between intraspecific lineages. We detected reticulated evolutionary patterns among lineages with different morphologies, resulting in a putative recent hybrid. Our study shows that phylogenomic analyses of unlinked nuclear loci, complemented with morphometrics, can resolve complex evolutionary histories of recently diverged species complexes.

# Presence and evolution of DNA methyltransferases and associated domains in brown algal genomes

Khatei, A.<sup>1</sup>, Cock, J.M.<sup>2</sup>, Tirichine, L.<sup>3</sup> & Jueterbock, A.<sup>1</sup>

<sup>1</sup>NORD Universitet

#### THURSDAY 23<sup>rd</sup> August 2023 Room « Tali ebrel »

 <sup>2</sup>Algal Genetics Group, Integrative Biology of Marine Models Laboratory, CNRS, Sorbonne Université, Station Biologique de Roscoff, CS 90074, F-29688 Roscoff, France
 <sup>3</sup>Nantes Université, CNRS, US2B, UMR 6286, F-44000 Nantes, France ananya.khatei@nord.no

DNA methylation is a widely studied epigenetic mechanism because of its importance in gene regulation. Moreover, DNA methylation can increase adaptive capacity beyond genetic potential and its influence on transgenerational plasticity may determine the evolutionary capacity of species. While DNA methylation has been shown to be present in the kelp Saccharina japonica, it was not found in the filamentous alga *Ectocarpus* sp. In this study, we aimed to bioinformatically annotate the presence and evolution of DNA methyltransferases (DNMTs) in 20 novel brown algal genomes as an indirect measure for the potential of these species to use DNA methylation as epigenetic mechanism. In other eukaryotes, DNA methyltransferases (DNMTs) catalyze the methylation of DNA cytosines. Previously, only DNMT2 has been found in the genomes of Saccharina japonica and Ectocarpus, an enzyme that is generally involved in tRNA methylation rather than DNA methylation. While we found DNMT2 in all 20 species our study suggests that DNMT1, a maintenance DNA methyltransferase, was lost early in the phylogenetic tree of brown algae. Interestingly, certain methyltransferase domains were found similar to DNMTs that could be contributing to DNA methylation in brown algae. Our study provides a first indication that DNA-methylation in brown algae is catalyzed either by methyltransferases that catalyze rather RNA methylation in plants and animals, or by other enzymes that are not direct homologues of the *de novo* or maintenance DNA methyltransferases in other eukaryotes. This study provides novel insights into the epigenetic mechanisms and evolution in brown algae.

### Fucoxanthin-Containing Dinoflagellates: An Open Experiment in Plastid Proteome Evolution

Novak Vánclová, A.M.G.<sup>1</sup>, Nef, C<sup>1</sup>., Füssy, Z.<sup>2</sup>, Váncl, A.<sup>2</sup>, Liu, F.<sup>1</sup>, Richardson, E.H.<sup>3,4</sup>, Dacks, J.B.<sup>4</sup>, Howe, C.J.<sup>3</sup>, Bowler, C.<sup>1</sup> & Dorrell, R.G.<sup>1,3</sup>

<sup>1</sup>CNRS, France <sup>2</sup>Charles University Prague, Czech Republic <sup>3</sup>University of Cambridge, UK <sup>4</sup>University of Alberta, Canada richard.dorrell.algae@gmail.com

Dinoflagellates are renowned not only for their ecological importance, but also for their divergent and unusual cell biology. Within the chloroplast, this includes genome organization features (minicircles) and gene expression pathways (polyU tail addition, extensive RNA editing) unique to dinoflagellates; and repeated secondary losses of photosynthesis, and chloroplast replacements via serial endosymbiosis, across the dinoflagellate tree. For example, the fucoxanthin-containing chloroplasts of the HAB-forming dinoflagellates *Karenia, Karlodinium* and *Takayama*, which have originated through a replacement of the ancestral peridinin-containing dinoflagellate plastid with a new organelle of haptophyte origin. My group has been studying the post-endosymbiotic trajectory of the fucoxanthin dinoflagellate chloroplast for a decade, focusing on changes to its genome and associated nucleus-encoded proteome

chloroplast for a decade, focusing on changes to its genome and associated nucleus-encoded proteome relative to free-living haptophytes. We have shown that the fucoxanthin chloroplast is an evolutionary hybrid, using chloroplast-associated pathways derived both from the haptophyte endosymbiont and the peridinin plastid previously present in the dinoflagellate host. The dramatic evolution of the fucoxanthin

chloroplast proteome has been accompanied by a phenomenal divergence and fragmentation of its genome.

Most recently, we have used transcriptomics, *in silico* targeting prediction, phylogenomics and environmental modeling to reconstruct the holistic evolution of the *pan*fucoxanthin chloroplast proteome. These include pinpointing a probable origin of the fucoxanthin chloroplast within the haptophyte order Chrysochromulinales, and evidence that its endosymbiotic uptake by the dinoflagellate host was accompanied by striking changes in ecological niche. Finally, we show that the phylogenetic composition of the fucoxanthin chloroplast proteome varies not only across different pathways proteins but even varies between species, underpinning the importance of micro-evolutionary processes for chloroplast functions across the algal tree of life.

### Sequencing of seaweeds ancient DNA from Monte Verde, one of the oldest archeological sites from South America

Guillemin, M-L.<sup>1,2</sup>, Valero, M.<sup>1</sup>, Mauger, S.<sup>1</sup>, Faugeron, S.<sup>1,3</sup>, Destombe, C.<sup>1</sup>, Utge, J.<sup>4</sup>, Verdu, P.<sup>4</sup>, Bon, C.<sup>4</sup>, Corre, E.<sup>5</sup>, Le Corguillé, G.<sup>5</sup> & Dillehay, T.D.<sup>6,7,8</sup>

<sup>1</sup>IRL 3614 Evolutionary Biology and Ecology of Algae, CNRS, Sorbonne Université, Pontificia Universidad Católica de Chile, Universidad Austral de Chile, Roscoff, France
<sup>2</sup>Núcleo Milenio MASH, Universidad Austral de Chile, Valdivia, Chile
<sup>3</sup>Núcleo Milenio MASH, Pontificia Universidad Católica de Chile, Santiago, Chile
<sup>4</sup>UMR7206 Eco-anthropologie, CNRS-MNHN-Université Paris Cité, Paris, France
<sup>5</sup>UPMC, CNRS, FR2424, ABiMS, Station Biologique de Roscoff, Roscoff, France
<sup>6</sup>Department of Anthropology, Vanderbilt University, Nashville, USA
<sup>7</sup>Escuela de Arqueologia, Universidad Austral de Chile, Puerto Montt, Chile
<sup>8</sup>School for Advanced Research, Santa Fe, New Mexico, USA
marielaure.guillemin@gmail.com

Fleshy algae are rarely preserved in archaeological sites and represent phyla evolutionarily distant from those usually studied using ancient DNA extraction and sequencing methods. Here we report the results of massive sequencing of ancient DNA extracted from a masticated cud formed by a mixture of algae, probably used as medicine or food supplement some 14,500 years ago in the archaeological site of Monte Verde, located in the south-central Chile (Dillehay et al. 2008). The 250 million reads obtained were aligned on a reference genome containing 60 brown and 24 red algae for which 1) a draft genome was available or 2), in the case of the genera listed as present at Monte Verde based on morphology for which no draft genome exist, the genetic data available. Consistent with the sample's old age, sequences showed the typical signatures of nucleotide misincorporation and DNA fragmentation observed for ancient DNA. Alignment results well supported the algal diversity detected previously based on morphological criteria at Monte Verde, with sequences of *Macrocystis, Sargassum, Durvillaea, Porphyra, Gracilaria, Mazzaella*, and *Sarcopeltis* retrieved in our sample. Genetic data also detected some new genera such as *Pyropia, Ectocarpus* and *Pylaiella* as potentially part of the seaweeds present in Monte Verde. Our results provide for the first time a catalogue of partial genomes of Pleistocene algae that highlights the surprising diversity of seaweeds used by early human communities in South America.

### Uncovering local adaptation to temperature process in the red seaweed *Palmaria palmata* intertidal populations

Baud, A.<sup>1,2</sup>, Fraumont, L.<sup>3</sup>, Potin, D.<sup>2</sup>, Valero, M.<sup>1</sup>, Potin, P.<sup>2</sup> & Destombe, C.<sup>1</sup>

<sup>1</sup>IRL EBEA 3614, Evolutionary Biology and Ecology of Algae, CNRS, Sorbonne Université, Pontificia Universidad Católica de Chile, Universidad Austral de Chile, Station Biologique de Roscoff, CS 90074, Place Georges Teissier, 29688 Roscoff cedex

<sup>2</sup>UMR8227 Integrated Biology of Marine Models, Station Biologique de Roscoff, France

<sup>3</sup>Université de Caen Normandie, CS 14032 14000 CAEN

aurelien.baud@sb-roscoff.fr

*Palmaria palmata* is a stenothermous red seaweed from the lower intertidal zone found in cold temperate and arctic waters of the North Atlantic. Due to its location on the shore, this species is higly exposed to warming of coastal sea surface waters induced by climate change. In this study, we focused on natural populations of *Palmaria palmata*, located along the Brittany coast, which are more or less exposed to temperature changes. Indeed, in *situ* temperature monitoring over 2 years reavealed that populations in the North-East of Brittany were exposed to greater annual variations than those in North-West. Furthermore, genetic analysis of these populations, based on 11 microsatellite DNA markers, showed that they were genetically differentiated. To test the difference between populations, a common garden experiment was conducted using individuals from four sites (two from north-west and two from northeast of Brittany). The response and recuperation to heat stress (5 days at 22.5 °C) was carried out by measuring photosynthetic efficiency of vegetative tissue using PAM (Pulse Amplitude Modulation) fluorometry. Our results show contrasting differences in the physiological response to stress between eastern and western populations, suggesting an ongoing local adaptation process within this species.

### Fine-scale genetic structure of sugar kelp populations at short geographical distance

Bråtelund, S.<sup>1</sup>, Ruttink, T.<sup>2,3</sup>, Goecke, F.<sup>1</sup>, Klemetsdal G.<sup>1</sup>, Ødegård, J.<sup>1</sup> & Ergon, A.<sup>1</sup>

<sup>1</sup>Norwegian University of Life Sciences, Faculty of Biosciences, Norway <sup>2</sup>Plant Sciences Unit, Flanders Research Institute for Agriculture, Fisheries and Food (ILVO), Belgium <sup>3</sup>Department of Plant Biotechnology and Bioinformatics, Ghent University, 9052 Ghent, Belgium signe.bratelund@nmbu.no

Sugar kelp (*Saccharina latissima*) is an important kelp species in Europe, not just ecologically, but lately also commercially. In Europe, the kelp cultivation industry is growing fast, and it is therefore essential to obtain new knowledge about the genetics of this species, both with future breeding in mind and to assess ecological risk such as genetic admixture with wild populations. We sampled 151 wild individuals from 10 locations within a radius of 10 km outside Trondheim, Norway. Genotyping-by-sequencing (GBS) identified 20710 SNPs and 12012 short haplotype loci within 20297 sequenced loci, across the sample set. Genetic diversity and population substructure were analyzed based on either SNPs or short haplotypes and by including or excluding rare alleles. Global  $F_{ST}$  values showed small, but significant, subdivision in the population and significant pairwise  $F_{ST}$  values were found between subpopulations that were located only a few kilometers apart.  $F_{IS}$  values showed a low but significant level of outbreeding. Genomic relationship matrices (GRMs) were constructed with SNPs or short haplotypes. Hierarchical clustering of the GRMs revealed differentiation between subpopulations. The main findings

were coherent across all datasets, however, the resolution increased by excluding rare alleles and by using multi-allelic short haplotypes compared to bi-allelic SNPs. The 151 individuals sampled in this study were used as the parent generation in a short-term selection experiment. Analysis of genetic transmission between the generations in this selection experiment showed that a small subset of parental lines was dominating in the offspring.

#### Genus-wide transcriptional responses to short-term warming in eastern Atlantic *Laminarias* (Phaeophyceae, Laminariales): a triumph of phylogeny over phenotype?

Martins, N.<sup>1</sup>, Madeira, P.<sup>1</sup>, Serrão, E.A.<sup>1</sup>, Bartsch, I.<sup>2</sup> & Pearson, G.A.<sup>1</sup>

<sup>1</sup>Centre of Marine Sciences (CCMAR), Faro, Portugal <sup>2</sup>Alfred-Wegener-Institute, Bremerhaven, Germany gpearson@ualg.pt

An important question to understand adaptive responses to climate change is whether there are common molecular phenotypes for thermal responses across recently diverged species groups. We aimed to address this question for foundation species that form marine forests, by undertaking a comparative analysis of five species of *Laminaria* with differing thermal affinities from the eastern Atlantic: *L. solidungula* (Arctic), *L. digitata*, *L. hyperborea* (both cold temperate), *L. ochroleuca* and *L. pallida* (both warm temperate). F1 sporophytes cultured *in vitro* were exposed for 7 days to temperatures between zero (*L. solidungula* only) and 22°C. Physiological indicators (growth, Fv/Fm) were recorded and tissue samples (at 5, 18 and 22°C) were taken at the end for RNAseq.

Growth responses confirmed the thermal affinities of each species. RNAseq data were used for *de novo* transcriptome reconstruction, gene-based comparative expression, weighted gene correlation network analysis (WGCNA), and phylogenomic analysis based on single copy orthologues. Species trees show *L. solidungula* diverging prior to a split between the sister clades *L. digitata/L. hyperborea* and most recently *L. ochroleuca/L. pallida*. Expression-based clustering strongly reflected phylogenomic divergence while thermal responses played a comparatively minor role. Similarly, WGCNA revealed several speciesspecific gene modules, but none related to thermal exposure. Common "core" sets of genes responding to temperature across all species were very small or absent but were larger within "thermal clades" (*L. digitata/L. hyperborea* or *L. ochroleuca/L. pallida*). The results suggest that many genes responding to thermal stress may show rapid turnover rates during diversification and speciation.

#### Scarlet tide - the first report of sex chromosomes in red algae

Lipinska, A.P.<sup>1,2#</sup>, Cossard, G.<sup>1,2#</sup>, Epperlein, P.<sup>1</sup>, Krueger, S.<sup>3</sup>, Godfroy, O.<sup>2</sup>, Ayres, L.<sup>4</sup>, Mauger, S.<sup>5</sup>, Scornet, D.<sup>2</sup>, Marchi, F.<sup>4</sup>, Lavaut, E.<sup>5</sup>, Destombe, C.<sup>5</sup>, Plestino, E.<sup>4</sup>, Oliveira, M.<sup>4</sup>, Barrera-Redondo, J.<sup>1</sup>, Guillemin, M.L.<sup>5,6,7</sup>, Valero, M.<sup>5</sup> & Coelho, S.M.<sup>1</sup>

<sup>1</sup>Department of Algal Development and Evolution, Max Planck Institute for Biology Tübingen, Germany <sup>2</sup>Sorbonne Université, CNRS, Integrative Biology of Marine Models, Station Biologique de Roscoff, France <sup>3</sup>Department of Biology, University of Alabama at Birmingham, USA

<sup>4</sup>Departamento de Botânica, Instituto de Biociências, Universidade de São Paulo, Brasil.

<sup>5</sup>CNRS, Sorbonne Université, Pontificia Universidad Católica de Chile, Universidad Austral de Chile, IRL 3614, Evolutionary Biology and Ecology of Algae, Station Biologique de Roscoff, France

#### THURSDAY 23<sup>rd</sup> August 2023 Room « Tali ebrel »

<sup>6</sup>Núcleo Milenio MASH, Facultad de Ciencias, Instituto de Ciencias Ambientales y Evolutivas, Universidad Austral de Chile, Valdivia, Chile <sup>7</sup>Centro FONDAP de Investigación de Ecosistemas Marinos de Altas Latitudes (IDEAL), Valdivia, Chile alipinska@tuebingen.mpg.de

Red algae (Rhodophyta) belong to the oldest evolutionary lineages of photosynthetic eukaryotes and comprise one of the largest phyla of algae on the planet. Most of the red algal species are multicellular and macroscopic, live in marine environment and have a complex life history with alternation of three, rather than two, generations. Particularly interesting are the aspects of the evolution of sex determination during the haploid life stage (UV sex chromosomes), however, the sex chromosomes in Rhodophyta have never been described to date. In this study, we used high-quality genomic, transcriptomic and genetic marker data to provide the first report of the red algal UV sex chromosomes using three species from the order Gracilariales. We report the genomic architecture and gene content of the male (V) and female (U) sex chromosomes and their nonrecombining regions. The UV system in Gracilariales shows distinct evolutionary history not only from the well-studied XY and ZW systems but also from the other algal UV systems described so far. Nevertheless, some striking similarities exist, indicating remarkable universality of the underlying processes shaping sex chromosome evolution across major eukaryotic supergroups.

### SYMPOSIUM 04 "Algae and their intimate partners"

#### **KEYNOTE**

# Vitamin B<sub>6</sub> derivatives mediate symbiotic exchanges between microalgae and bacteria

Amin, S.A.<sup>1</sup>, Ocshenkühn, M.A.<sup>1</sup>, Coe, L.S.Y.<sup>1</sup>, Littman, S.<sup>2</sup>, Tienken, D.<sup>2</sup> & Mohamed, A.R.<sup>1</sup>

<sup>1</sup>Marine Microbiomics Lab, New York University Abu Dhabi, United Arab Emirates <sup>2</sup>Max-Planck Institute for Marine Microbiology, Germany samin@nyu.edu

Symbiotic relationships between microalgae and bacteria play a crucial role in the functioning of aquatic ecosystems as they influence nutrient cycling and carbon biogeochemistry. These relations are governed by microscale interactions that play out in a small region surrounding microalgal cells, known as the phycosphere. The phycosphere is enriched in microalgaederived organic molecules that help attract, select and nurture beneficial microbiomes in the phycosphere. Despite the importance of these relations, the communication mechanisms governing these symbioses remain poorly understood.

Here, we use an <sup>15</sup>N-labeled diatom *Asterionellopsis glacialis* and members of its microbiome to show that only symbiotic bacteria can assimilate diatom-derived dissolved organic nitrogen (DON). Using novel mass spectrometry techniques, we simultaneously identify and quantify ~200 secreted diatom DON metabolites of which >60 metabolites are concomitantly taken up by its symbiotic bacteria. Among these the vitamin B<sub>6</sub> derivative, 4pyridoxolactone, was taken up by bacteria, which in return converted it to 4-pyridoxate that the diatom took up. Using transcriptomics and gene network analyses we show that 4pyridoxate triggers regulation of hundreds of genes in the diatom to promote DON secretion, while 4-pyridoxolactone primes the bacteria to take up and catabolize specific diatom-derived DON.

The discovery of vitamin  $B_6$  intermediates that function as interkingdom signaling molecules is significant as these intermediates have no known biological function in nature. These findings provide novel and significant insight into symbiosis between microalgae and their bacterial partners and highlight the influence of signaling on major biogeochemical cycles.

# To what extent and how does the phytoplankton choose their bacterial partners? A reductionist approach used in toxic microalgae

Garnier, M., Patron, L. & Briand, E.

*IFREMER, PHYTOX, GENALG, France* matthieu.garnier@ifremer.fr

Biotic interactions within the phycosphere are one of the main drivers of the functionality of phytoplankton from cellular to global scales. To what extent do the microalgae drive the assembly of the bacterial community within the phycosphere, and what are the consequences on the functionality of the phytoplankton are important issues. Here, we developed a reductionist approach, and gradually increasing the biological complexity to study the assembly of bacterial communities within the

phycospheres of the toxic dinophyte Alexandrium minutum and the ichtyotoxic haptophyte *Prymnesium* parvum.

The monitoring of 12 selected bacteria grown in the phytoplankton extracellular fraction (PEF) indicated that they all were able to grow with FEP of both algae as sole source of nutrient. The bacteria were mixed in synthetic community (SynCom) and cultured on PEF. PEF allowed the growth of copiotrophic strains, irrespective of the producing alga. However the exometabolites of the two species led to distinct populations with some of algae specific growing bacteria. SynCom and microalgae were then cocultured in photobioreactors. Very early, algae-specific communities of bacteria were observed with a great correlation of communities with cultures on PEF. Overall, our results obtained in carefully controlled conditions support the assertion that the phytoplankton cells select the bacteria that surround them though the production of specific exo-metabolites. What are these metabolites involved and how these selected bacteria impact the resilience of phytoplankton to different nutrient deficiency conditions? These are the questions we are now addressing.

# Viral impacts across biological scales: from single-cells to planktonic ecosystems

Vincent, F.<sup>1</sup> & Vardi, A.<sup>2</sup>

<sup>1</sup>Developmental Biology Unit, European Molecular Biological Laboratory, Germany <sup>2</sup>Plant and Environmental Sciences Department, Israel flora.vincent@embl.de

Marine viruses are the most abundant entity in the ocean but their impact on microbial dynamics and biogeochemical fluxes remains unresolved. By assessing active viral infection within single cells of the blooming alga *Emiliania huxleyi* using mRNA *in situ* hybridization (Vincent et al., 2021), we revisit its giant virus life cycle and suggest potential host defense strategy by quantifying biomass aggregation. We further explore the impact of *E. huxleyi* blooms on microbial communities and biogeochemical processes, during a large-scale mesocosm experiment (Vincent\*, Gralka\*, et al., 2023). With only 25% of the cells infected in a natural bloom, giant virus infection can modulate the ratio between prokaryotic and eukaryotic recyclers of organic matter while boosting carbon exudation from infected single cells. Combined with single cell transcriptomics on natural samples (Hevroni\*, Vincent\* et al., 2023), we further expand marine microbiology's foundational concepts of the microbial loop and viral shunt. Overall, our work aims to demonstrate the conceptual power of tracing intimate microbial interactions across different biological scales, from lab-based controlled experiments to large scale environmental sampling expeditions (Vincent & Vardi, 2023).

# SYMPOSIUM 05 "New insights into the mechanisms and regulation of life cycles in algae"

#### **KEYNOTE**

# Multi-species comparative -omics reveals marker genes for the diatom life cycle

#### Bilcke, G.<sup>1,2,3</sup>

<sup>1</sup>VIB Center for Plant Systems Biology, Technologiepark 71, 9052, Ghent, Belgium

<sup>2</sup>Department of Plant Biotechnology and Bioinformatics, Ghent University, Technologiepark 71, 9052, Ghent, Belgium

<sup>3</sup>*Protistology and Aquatic Ecology, Department of Biology, Ghent University, 9000, Ghent, Belgium* Gust.Bilcke@ugent.be

Diatoms, the most productive and species-rich group of unicellular algae, have a sophisticated life cycle in which the cell size gradually decreases with successive vegetative divisions. Typically, cell size can only be restored through the formation of an auxospore during sexual reproduction. Yet, observations of sexual events in nature are infrequent and the phenology of auxosporulation remains poorly understood.

Between ~1500 and ~9000 genes were differentially expressed during sexual reproduction in four diatom species, including the benthic *Seminavis robusta* and *Cylindrotheca closterium* and the planktonic *Skeletonema marinoi* and *Pseudo-nitzschia multistriata*. A comparative transcriptomics analysis using gene orthology and homologous gene families from the PLAZA Diatoms platform, as well as detailed phylogenetic tree analysis, revealed dozens of genes showing conserved upregulation. Among these, several conserved transcription factors were found, suggesting these genes represent important regulators of sex in diatoms. Furthermore, the different cell types (gametangia, gametes, zygotes and auxospores) captured by these time series transcriptomes permitted the identification of genes associated with specific stages during this developmental process.

We used our integrated dataset to identify a panel of candidate sex marker genes, which we validated by RT-qPCR analyses of laboratory crosses and applied to detect sexual events in a metatranscriptomic experiment of a natural phytoplankton community as well as 68 stations derived from the Tara Oceans expedition. Taken together, these markers offer a novel costeffective method to track life cycle progression in diatom populations, and further improve our understanding of the ecological success of diatoms and their adaptation to changing environments.

### ORALS

# Multiomics responses to seasonal variations in diurnal cycles in the marine picoeukaryote *Ostreococcus tauri*

Romero-Losada, A.B.<sup>1,2</sup>, Arvanitidou, C.<sup>1,2</sup>, García-Gómez, M.E.<sup>1</sup>, Morales-Pineda, M.<sup>1</sup>, García-González, M.<sup>1</sup> & Romero-Campero, F.J.<sup>1,2</sup>

<sup>1</sup>Institute for Plant Biochemistry and Photosynthesis, Universidad de Sevilla – Consejo Superior de Investigaciones Científicas; Av. Américo Vespucio 49, 41092 Seville (Spain) <sup>2</sup>Department of Computer Science and Artificial Intelligence, Universidad de Sevilla; Av. Reina Mercedes s/n, 41012 Seville (Spain) fran@us.es

Earth tilted rotation and translation around the Sun produce periodic environmental signals giving rise to seasonal variations in diurnal cycles. This has promoted the emergence during evolution of cellautonomous molecular oscillators known as circadian clocks that predict and anticipate these changes. Although marine phytoplankton plays a key role on ecosystems and present promising biotechnological applications, multiomics integrative analysis of their response to these rhythms remains largely unexplored. We have chosen the marine picoeukaryote Ostreococcus tauri as model organism due to its cellular simplicity, availability of its fully sequenced and annotated genome and abundance in marine ecosystems. We have integrated RNA-seq, ChIP-seq, SWATH-MS proteomics and physiological data generated under summer long day conditions (16h light:8h dark) and winter short day conditions (8h light:16h dark). Free running conditions consisting of constant light and constant dark were also included. Although 80% of the transcriptome presented diurnal rhythmicity under both seasonal conditions less than 20% maintained oscillations under constant conditions. A drastic reduction in protein abundance rhythmicity was observed with only 34% of the proteome oscillating. Seasonally specific rhythms were found in key physiological processes such as photosynthetic efficiency, cell cycle progression, carotenoid content, starch accumulation and nitrogen assimilation. A global orchestration between transcriptome, proteome and physiological dynamics was detected with specific seasonal temporal offsets between transcript, protein and physiological peaks. To further explore this we determined the gene targets of Ostreococcus CCA1 (Circadian Clock Associated 1) ortholog at dawn using ChIPseq data.

### Role of the maternal tissue in the establishment of polarity axes in the early embryo of the brown alga Saccharina latissima

Boscq, S., Billoud, B., Joemmanbacks, T. & Charrier, B.

Morphogenesis of Macro Algae, UMR8227, CNRS - Sorbonne university, Station Biologique de Roscoff, France samuel.boscq@sb-roscoff.fr

In all organisms, embryo spatial axes are the foundation of the developing body organization up to the adult stage. Currently, in brown algae almost nothing is known of the parental influence on development. *Saccharina latissima*, from the Laminariales group, is one of the few brown algae to remain connected to its maternal tissue during development. It develops a linear stack of 8 cells from parallel transverse divisions of the zygote while remaining connected to the stalk (husk of the dead oogonium) which sets the apico-basal axis of the embryo which remains the main body axis at the adult stage (up to 3m).

We developed microdissection and laser ablation protocols to ablate the connection to the maternal tissue and specific cells of the embryo at different stages of development (Boscq et al., Jove 2022) and monitored the embryo for two weeks. Using a home-coded software we extracted at all time points morphological data (cell orientation, number of neighbors, blade and cells area and shape descriptors). We showed that embryos disconnected from their maternal tissue present loss of axis establishment, premature widening of the blade, a disorientation of cells and a local division rate increase. Ablation of the basal cell of the developing embryo shows similar results. We also observed that ablation of the apical cell results in reduced growth rate showing the most of the embryo's elongation occurs at its apex. Together these are the first results toward a functional role of the maternal tissue in brown algae embryogenesis.

#### KEYNOTE

### Zygote (a)symmetric cell division in *Dictyota*: a developmental hinge point

Bogaert, K.A.<sup>1,2</sup>, Zakka, E.E.<sup>1</sup>, Coelho, S.M.<sup>2</sup>, Beeckman, T.<sup>3</sup> & De Clerck, O.<sup>1</sup>

<sup>1</sup>Phycology Research Group, Biology Department, Ghent University

<sup>2</sup>Department of Algal Development and Evolution, Max Planck Institute for Biology Tübingen, Germany

<sup>3</sup>Vlaams Instituut voor Biotechnologie-Ghent University Center for Plant Systems Biology, Ghent, Belgium kenny.bogaert@tuebingen.mpg.de, kenny.bogaert@ugent.be

Zygote development serves as a pivotal point in the life cycle, connecting the gametophytic and sporophytic life stages and playing a crucial role in the recruitment of the next generation of seaweeds. Broadcast spawning brown algae are excellent models for studying processes during the life cycle transition, asymmetric cell division, and attachment because of their synchronous development free from maternal tissue.

In the oogamous brown alga *Dictyota dichotoma*, early development has been described. The sporophytic developmental program is rapidly activated following fertilization, unlike in animal systems. After 1 to 2 minutes of rapid elongation, the zygote undergoes asymmetric cell division, establishing the apical-basal pattern, with the elongated zygote developing into the rhizoid responsible for seaweed recruitment. In addition, basal cell fate differentiation in *Dictyota* shows a remarkable degree of phenotypic plasticity and may be postponed under the control of an endogenous volatile compound. The ability to control asymmetric cell division versus symmetric cell division enables the comparison of a polarized zygote with an unpolarized zygote in the same developmental context. Using cell polarization assays combined with transcriptomics, we describe the phenotypic modulation of asymmetric cell division.

### Transcriptome differentiation between life phases of the bloom forming marine phytoplankton specie *Emiliania huxleyi*

Bousquet, L.<sup>1,2</sup>, Fainsod, S.<sup>1,2</sup>, Murik, O.<sup>2</sup>, Decelle, J.<sup>3</sup>, Avrahami, Y.<sup>1,2</sup>, Koplovitz, G.<sup>1,2</sup> & Frada, M.J.<sup>1,2</sup>

<sup>1</sup>The Interuniversity Institue for Marine Sciences of Eilat, Israel <sup>2</sup>Hebrew University of Jerusalem, Israel <sup>3</sup>Univ. Grenoble Alpes, France laurie.bousquet@mail.huji.ac.il The cosmopolitan coccolithophore *Emiliania huxleyi* is a unicellular eukaryotic algae responsible for reoccurring large oceanic blooms serving as the foundation of marine food webs. *E. huxleyi* is characterized by a heteromorphic and haplo-diplontic life cycle. While most studies focus on the diploid calcified, non-motile and bloom forming life-phase, the noncalcified and bi-flagellated haploid phase has been overlook. In addition to their heteromorphy, they display utterly different responses to infection by the lytic *E. huxleyi* virus (Ehv) where haploid cells are insensitive to infection. Following *in-vitro* infection, a new diploid population emerges, displaying a haploid phenotype that is bi-flagellated, noncalcified and resistant to Ehv., hereby referred to as decoupled This decoupling could provide an adaptive escape strategy from Ehv infection.

To investigate the mechanisms underlying this switch in life-phase and subsequent resistance to infection, we conducted a comparative study on these three life-phases of *E. huxleyi*. We generated a unique "Life-Phase transcriptome" of calcified, haploid and decoupled cells. Quantitative expression analysis coupled with functional annotation, highlighted that expression patterns of haploid and decoupled cells are similar relative to calcified cells (85% of the variance explained within PCA). In addition to a set of shared core transcripts, all life-phases contained unique transcripts involved in distinct cellular processes, including putative epigenetic mechanisms.

These results pave the way for further *in-vivo* studies of *E. huxleyi* life cycle as well as under viral infection.

#### Developmental and evolutionary surveys of gene expression during brown algal life cycles

Ratchinski, P.<sup>1</sup>, Godfroy, O.<sup>1</sup>, Lipinska, A.<sup>2</sup>, Bogaert, K.<sup>3</sup>, Scornet, D.<sup>1</sup>, Dartevelle, L.<sup>1</sup>, Peters, A.F.<sup>4</sup>, Cruaud, S.<sup>5</sup> & Cock, J.M.<sup>1</sup>

<sup>1</sup>Station Biologique de Roscoff, Sorbonne Université, CNRS, Algal Genetics Group, Integrative Biology of Marine Models (LB12M), Station Biologique de Roscoff (SBR), France
<sup>2</sup>Department of Algal Development & Evolution - Max Planck Institute for Biology, Germany
<sup>3</sup>Phycology Research Group, UGent., Belgium
<sup>4</sup>Bezhin Rosko, 29250, Santec, France
<sup>5</sup>CEA, DSV, Institut de Génomique, Génoscope, France
pelagie.ratchinski@sb-roscoff.fr

Phaeophyceae (brown algae) is a clade of eukaryotic multicellular photosynthetic organisms phylogenetically very distant from the well-studied green lineage. Most brown algal species alternate a diploid sporophytic and a haploid gametophytic phase during their life cycle. However, generation identity is independent of ploidy as, for example, haploid sporophytes can develop through parthenogenesis. Previous studies, in the model species *Ectocarpus species 7*, identified genes that control the developmental program of the sporophyte generation. In two nearly isomorphic model species *Ectocarpus species 7* and *Dictyota dichotoma*, we used transcriptomic data corresponding to different developmental timepoints to analyse gene co-expression during the life cycle. Furthermore, while most brown algal species possess two life cycle generations, the relative importance and complexity of these generations varies across the brown algal phylogenetic tree. To determine whether generationbiased genes are conserved among brown alga, we analysed RNAseq data from adult stages of ten phylogenetically diverse brown algal species with a high diversity of life cycles.

### Unveiling mechanisms of sexual differentiation using feminized males of the giant kelp *Macrocystis pyrifera*

Liesner, D.<sup>1</sup>, Haas, F.B.<sup>1</sup>, Barrera-Redondo, J.<sup>1</sup>, Müller, D.G.<sup>2</sup>, Zheng, M.<sup>1</sup>, Godfroy, O.<sup>3</sup> & Coelho, S.M.<sup>1</sup>

<sup>1</sup>Department of Algal Development and Evolution, Max Planck Institute for Biology Tübingen, Germany <sup>2</sup>Department of Biology, University of Konstanz, Germany <sup>3</sup>Integrating Biology of Maring Models, Station Biologiana de Bossoff, England

<sup>3</sup>Integrative Biology of Marine Models, Station Biologique de Roscoff, France

daniel.liesner@tuebingen.mpg.de

In most brown algae, sex is determined in the haploid gametophyte stage by a female U and a male V chromosome, but sexually dimorphic traits are to a large extent a result of differential expression of (autosomal) genes that are present in both sexes. To understand the mechanisms underlying sexual differentiation in brown algae, we compared wild type and three feminized male mutant strains of the giant kelp *Macrocystis pyrifera* using morphometric, genomic and transcriptomic approaches.

Morphometric analysis of mutant lines revealed strong signs of feminization in these genetically male individuals, including increased gametophyte cell size. Upon fertility, feminized males produced gametangia morphologically similar to oogonia which released immotile gametes. These were significantly larger ( $\leq 500\%$ ) than male sperm, but significantly smaller ( $\leq 52\%$ ) than female eggs, and were capable of parthenogenesis, which is a female-specific trait in kelps.

The phenotypic feminization of these mutants was underlain by conspicuous transcriptome feminization (increased expression of female-biased genes) and de-masculinization (decreased expression of malebiased genes) compared to wild type males, concurrent with significantly reduced expression of a subset of male sex-linked genes. Remarkably, comparison of transcriptomes revealed that distinct sex-linked and autosomal genes underlie feminization in the different mutant lines, suggesting that diverse, alternative pathways may lead to the same feminized phenotype in these genetically male individuals. A subset of genes, however, exhibited consistent changes in their pattern of expression associated with the feminization, and we will discuss how these genes may provide clues to further understand male versus female sexual differentiation.

### Sexual reproduction, a major event determining the dynamics of gene expression during a *P. australis* bloom?

Prigent, L., Quéré, J., Plus, M. & Le Gac, M.

Ifremer, DYNECO/PELAGOS, B.P. 70, 29280 Plouzané, France lea.prigent@ifremer.fr

Phytoplankton blooms are an important, widespread phenomenon in all aquatic ecosystems, supporting food webs and essential ecosystem services. Ecological studies have provided valuable insights about the link between environmental variables and species successions *in situ*. However, the *in situ* physiology of blooming species remains poorly characterized. This may now be addressed by using metatranscriptomic. During one month, in April 2017, a metatranscriptomic sampling was carried out during the bloom of the toxin producing species *Pseudo-nitzschia australis* in the Bay of Brest (Brittany, France). These samples were analyzed to investigate for the first time a harmful algae species and more generally a phytoplankton's species gene expression dynamics during the entire period of a bloom *in situ*. Using a global approach, several functional gene categories displayed a strong temporal pattern of differential gene expression. At the beginning of the bloom, genes involved in photosynthesis, energy

production, storage as well as in toxin's biosynthesis were highly expressed and to a lesser extend at the end of the bloom. In the middle of the bloom, an abrupt change in gene expression was identified at the time of highest *P. australis*'s relative abundances with a marked expression of genes related to sexual reproduction. Following this, genes involved in ribosome biogenesis, protein synthesis and cell division were overexpressed for a few days. Altogether, this study highlights key functions displaying dynamic expression *in situ* and illustrates how functions mainly studied *in vitro* may help understand *in situ* blooms.

# Transcriptional regulation of sexual maturation in *Cylindrotheca* closterium

Belisova, D.<sup>1,3</sup>, Bilcke, G.<sup>2</sup>, Audoor, S.<sup>1</sup>, Pargana, A.<sup>1</sup>, D'hondt, S.<sup>1</sup>, Sabbe, K.<sup>1</sup>, De Veylder, L.<sup>3</sup>, Vandepoele, K.<sup>2</sup> & Vyverman, W.<sup>1</sup>

<sup>1</sup>Research Group Protistology and Aquatic Ecology, Department of Biology, Ghent University, Belgium
 <sup>2</sup>Comparative Network Biology, VIB-UGent Center for Plant Systems Biology, Belgium
 <sup>3</sup>Cell Cycle, VIB-UGent Center for Plant Systems Biology, Belgium
 darja.stojanovova@ugent.be

Cells intimately monitor their size throughout the cell cycle to match the environmental and organismal context. In diatoms, however, an additional, unique size-sensing mechanism evolved, controlling sexual maturation and their cell size reduction-restitution cycle. Only cells smaller than a species-specific Sexual Size Threshold (SST) can engage in sexual reproduction when suitable conditions are met.

Here, we present a differential expression analysis of the raphid diatom *Cylindrotheca closterium*, a common tychoplanktonic species globally present in coastal waters. Transcriptome profiling of three biological replicates for both mating types (MT $\pm$ ) and size classes above and below the SST revealed that only 112 out of 24,633 annotated genes are differentially expressed. About half of these show a mating type-specific expression irrespective of size (52%), while the other half showed an expression bias towards cells above or below the SST (48%). This suggests that a small set of key genes is responsible for the sexual maturation and mating type activation of cells, while the expression of genes involved in basic metabolism remains virtually unchanged throughout the size reduction trajectory. Two *C. closterium* transcripts with a Leucine-rich repeat (LRR) domain share homology with the *Pseudo-nitzschia multistriata* MT- marker MRM2. Interestingly, these MRM2 homologs show inverse expression patterns in *C. closterium*, either being specific for small MT+ or MT- cells. Further comparative analysis revealed a similar duplication and expression divergence for *Seminavis robusta* MRM2. Beyond that, the sexual maturation 'regulon' of *C. closterium* appears to be largely composed of species-specific genes.

## Ca<sup>2+</sup> signaling during fertilization in the red alga *Bostrychia moritziana*: the role of calcium-dependent kinases in ROS signaling

Shim, E.<sup>1</sup>, Park, H.<sup>1</sup>, Zuccarello, G.C.<sup>2</sup>, Kim, G.H.<sup>1</sup>

<sup>1</sup>Department of Biology, Kongju National University, Korea <sup>2</sup>School of Biological Sciences, Victoria University of Wellington, Wellington, New Zealand eyshim0412@gmail.com

Reactive oxygen species (ROS) signaling plays a variety of important roles in biological systems. ROS produced as byproducts of metabolic processes are largely removed by intracellular antioxidant

#### THURSDAY 23<sup>rd</sup> August 2023 Room « Tali moan »

enzymes, while ROS for cell signaling are produced by membranelocated NADPH oxidase/respiratory burst oxidative homolog (RBOH) proteins. Recent studies have shown that ROS signaling mediates gamete fusion, migration of spermatial nuclei, and development of carposporophytes in red algae. Calcium influx is an important cue for the activation of NADPH oxidases, but little is known about the genes that mediate calcium signaling in red algae. We observed calcium influx into the reproductive cells of Bostrychia moritziana using Flu8 staining and identified five calcium-dependent kinases using transcriptome data. Quantitative PCR results showed that calcium-dependent tyrosine kinase (CDTK), calcium-dependent serine threonine protein kinase (CDSTK), and calcineurin B-like interacting protein kinases (CIPK) are involved in the fertilization process. CDTK and CDSTK were most highly expressed during fertilization especially at the time of gamete fusion and migration of the spermatial nucleus inside the trichogyne, while CIPK expression increased only during the early post-fertilization processes. Caffeine treatment inhibited the expression of the calcium kinases and ROS production. When ROS production was inhibited by the treatment with diphenyleneiodinium chloride (DPI) or scavenged using antioxidants, Flu8 staining disappeared, and the upregulation of CDTK and CDSTK was not induced during fertilization. These results show that signaling during fertilization and early post-fertilization processes in *B. moritziana* is regulated by a positive feedback loop between calcium and ROS signals.

### Genetic and epigenetic controls governing life cycle transitions in the planktonic diatom *Pseudo-nitzschia multistriata*

Ferrante, M.I.<sup>1,2</sup>, Ruggiero, A.<sup>1</sup>, Manfellotto, F.<sup>1</sup>, Mager, S.<sup>1</sup>, Gerber, T.<sup>3</sup>, Pan, L.<sup>3</sup>, Arendt, D.<sup>3</sup>, Sanges, R.<sup>4</sup>, Russo, M.T.<sup>1</sup> & Montresor, M.<sup>1</sup>

<sup>1</sup>Stazione Zoologica Anton Dohrn, Naples, Italy <sup>2</sup>National Institute of Oceanography and Applied Geophysics, Trieste, Italy <sup>3</sup>European Molecular Biology Laboratory, Heidelberg, Germany <sup>4</sup>International School for Advanced Studies- SISSA, Trieste, Italy mariella.ferrante@szn.it

Phytoplankton dynamics are regulated by external cues, mainly light and nutrients, as well as by biotic interactions and endogenous controls linked to life cycle characteristics.

With few exceptions, sexual reproduction is an obligate phase in diatom life cycles, required to escape the miniaturization process imposed by the rigid cell wall, as well as to generate genetic diversity. The planktonic pennate diatom *Pseudo-nitzschia multistriata*, with a heterothallic mating system with two opposite mating types (MTs), represents a model for the study of diatom life cycles. Gene expression studies identified genes involved in the response to sexual clues and in the mechanism of sex determination.

How exactly sex is determined and what triggers competence for sex after a given cell size is reached are still open questions. To address these questions, we are using single cell technologies for the characterization of MT specific genes and the gene networks in which they participate, revealing different cell populations. We also performed a genome-wide Enzymatic Methyl-seq (EM-seq) analysis in cells of different size and of opposite MT to investigate the potential role of epigenetic controls in life cycle transitions. This approach allowed to describe the methylation landscape in *P. multistriata* and to compare the methylation profile in small and large cells, and in MT- and MT+. Our data show that the methylation pattern is stable over the *P. multistriata* life cycle but that the MT determination region is differentially methylated in MT- and MT+, suggesting a function for methylation in the sex determination process.

#### Isomorphic may not be so isomorphic after all

Krueger-Hadfield, S.A.<sup>1</sup>, Amsler, C.D.<sup>1</sup>, Bonthond G.<sup>2</sup>, DeForest J. <sup>3</sup>, Hamilton IV, W.<sup>4</sup>, Hays, C.G.<sup>5</sup>, Mieszkowska, N.<sup>6,7</sup>, Oetterer, A.P.<sup>1</sup>, Reed, K.B.<sup>8</sup>, Ryan, W.H.<sup>9</sup> & Thornton, B.M.<sup>1</sup>

<sup>1</sup>Department of Biology, University of Alabama at Birmingham1300 University Blvd, Birmingham, AL, 35294, USA

<sup>2</sup>Institute for Chemistry and Biology of the Marine Environment (ICBM), Carl von Ossietzky University Oldenburg, Schleusenstrasse 1, 26382, Wilhelmshaven, Germany

<sup>3</sup>Department of Environmental and Plant Biology, Ohio University, Athens, OH, USA, 45701

<sup>4</sup>Department of Forest Resources and Environmental Conservation, Virginia Tech, 310 W Campus Drive, Blacksburg, VA, 24060, USA

<sup>5</sup>Keene State College, 229 Main St., Keene, New Hampshire 03435

<sup>6</sup>The Marine Biological Association of the United Kingdom, The Laboratory, Citadel Hill, Plymouth, PL1 2PB, United Kingdom

<sup>7</sup>Department of Earth, Ocean and Ecological Sciences, University of Liverpool, England

<sup>8</sup>Department of Organismal and Environmental Biology, Christopher Newport University, One Avenue of the Arts, Newport News, VA 23606, USA

<sup>9</sup>Biology Department, Towson University, 8000 York Rd, Towson, MD, 21252 Department of Biology, University of Alabama at Birmingham1300 University Blvd, Birmingham, AL, 35294, USA

The diversity of lineages maintaining haploid-diploid life cycles suggests they are not transitory states, but evolutionarily stable. Here, we used the widespread, non-native, red macroalga *Gracilaria vermiculophylla* to test various forms of phenotypic differentiation between morphologically similar gametophytes and tetrasporophytes. Almost all softsubstratum habitats are dominated by tetrasporophytes, despite the likelihood that all life cycle stages are introduced to these habitats together. Here, we explore a suite of phenotypic variables in *G. vermiculophylla* which may help explain (1) the persistence of three superficially morphologically similar life stages at some sites and (2) the systematic loss of gametophytes at others. 'Isomorphic' female gametophytes, male gametophytes, and tetrasporophytes differ morphologically, in survival, in bacterial communities, and in combinations of different variables when analyzed as a 'composite' phenotype (e.g., thallus strength, protein, and photosynthetic pigments). Moreover, we have found evidence of ecotypic differentiation between fixed (i.e., attached by a holdfast to hard substrate) and freefloating (i.e., drifting) tetrasporophytes. We synthesize our available data to date to demonstrate that phenotypic differentiation is subtle – as predicted by Hughes and Otto in 1999 – and may not be captured accurately if the wrong phenotypes are investigated.

Moreover, we discuss the inadvertent confusion created by referring to these stages as 'isomorphic'. Understanding the convergence of ecology and genetics will allow us to test theoretical predictions about the evolutionary maintenance of the alternation of haploid and diploid stages (i.e., sex).

### SYMPOSIUM 09 "The global carbon-cycle"

### **KEYNOTE**

#### Carbon export from seaweed forests to deep ocean sinks

Filbee-Dexter, K.<sup>1,2</sup>, Pessarrodona, A.<sup>2</sup>, Pedersen, M.F.<sup>3</sup>, Wernberg, T.<sup>2</sup>, Duarte, C.M.<sup>4</sup>, Assis, J.<sup>5,6</sup>, Bekkby, T.<sup>7</sup>, Burrows, M.T.<sup>8</sup>, Carlson, D.F.<sup>9</sup>, Gattuso, J-P.<sup>10,11</sup>, Gundersen, H.<sup>7</sup>, Hancke, K.<sup>7</sup>, Krumhansl, K.A.<sup>12</sup>, Kuwae, T.<sup>13</sup>, Middelburg, J.J.<sup>14</sup>, Moore, P.J.<sup>15</sup>, Queirós, A.M.<sup>16</sup>, Smale, D.A.<sup>17</sup>, Sousa Pinto, I.<sup>18</sup>, Suzuki, N.<sup>19</sup> & Krause-Jensen, D.<sup>20</sup>

<sup>1</sup>Institute of Marine Research, Norway. <sup>2</sup>University of Western Australia; Australia. <sup>3</sup>Roskilde University, Department of Science and Environment. <sup>4</sup>King Abdullah University of Science and Technology (KAUST), Kingdom of Saudi Arabia. <sup>5</sup>Centre of Marine Sciences, University of the Algarve, Portugal. <sup>6</sup>Faculty of Bioscience and Aquaculture, Nord University, Norway. <sup>7</sup>Norwegian Institute for Water Research, Norway. <sup>8</sup>Scottish Association for Marine Science, UK. <sup>9</sup>Institute of Carbon Cycles, Helmholtz-Zentrum Hereon, Germany. <sup>10</sup>Sorbonne Université, France. <sup>11</sup>Institute for Sustainable Development and International Relations, France. <sup>12</sup>Fisheries and Oceans Canada, Canada. <sup>13</sup>Coastal and Estuarine Environment Research Group, Japan. <sup>14</sup>Department of Earth Sciences, Utrecht University, The Netherlands. <sup>15</sup>Dove Marine Laboratory, United Kingdom. <sup>16</sup>*Plymouth Marine Laboratory, Plymouth, United Kingdom.* <sup>17</sup>Marine Biological Association of the United Kingdom, Plymouth, UK. <sup>18</sup>Ciimar and Faculty of Sciences, Portugal. <sup>19</sup>Institute of Coastal Ocean Dynamics, Helmholtz-Zentrum Hereon, Germany. <sup>20</sup>Aarhus University, Department of Ecoscience, Denmark kfilbeedexter@gmail.com

The world's seaweed forests are the largest vegetated ecosystem in the ocean with per area productivity comparable to phytoplankton. Yet, the fate of the flux of carbon exported from seaweed habitats is a key unknown in the oceanic carbon budget and prevents accurate estimates of their potential for carbon storage. Here we create the first global spatially explicit estimate of seaweed particulate organic carbon (POC) export to the open ocean by combining models of seaweed forest distribution and net primary productivity (NPP) with seaweed decomposition and estimates of coastal shelf and open ocean exchange. The export potential of POC from seaweed forests across the shelf break (>200 m depth) is on average 16% of the annual NPP, or 35 g C exported m<sup>2</sup> seaweed forest y<sup>-1</sup>, which equates to 55 Tg C per year globally. There was high spatial variation, with the largest fluxes of seaweed carbon to the open ocean found in regions with narrow continental shelves, oceanic islands, submarine canyons, and large seaweed NPP. We also estimate that if this exported seaweed carbon is remineralized below 200 m depth, 65% will take over 25 years and 14% will take over 100 years to return to the sea surface. If exported seaweed carbon reaches greater depths, rates of return will be considerably slower, with 55%

of the carbon remineralized below 530 m depth being sequestered for over 100 years, which could be as much as 30 Tg C per year globally. These findings address a major knowledge gap on the fate of seaweed in the ocean carbon cycle, which has consequences for energy inputs to mesopelagic fisheries and estimates of overall carbon fluxes from ocean margins to ocean interior. Our results highlight a substantial potential contribution of seaweed to the biological pump, which to date has been largely ignored. Verifying associated carbon sequestration remains challenging yet critical given the high rates of seaweed forest loss and the increasing efforts to restore coastal ecosystems. Our results could contribute to international climate targets and may inform the possible climate change mitigation potential of actions to conserve and restore seaweed forests.

### ORALS

# Investigating the effects of allelopathy on organic matter sink during microalgal blooms

Long, M.<sup>1</sup>, Gesson, M.<sup>1</sup>, Walde, M.<sup>2</sup>, Guillou, L.<sup>2</sup> & Moriceau, B.<sup>1</sup>

<sup>1</sup>CNRS, Univ Brest, IRD, Ifremer, LEMAR, 29280 Plouzane, France <sup>2</sup>Sorbonne Univ, CNRS, Laboratoire Adaptation et Diversité en Milieu Marin UMR7144, Station Biologique de Roscoff, 29680 Roscoff, France marc.long@univ-brest.fr

Chemical communication represents the most frequent mode of interaction in the living organisms. Although this mode of interaction is key to ecosystem functioning, our understanding of these interactions among planktonic organisms is limited. Some photosynthetic microorganisms such as the dinoflagellate Alexandrium minutum that can inhibit competitors (interaction called "allelopathy") by releasing allelochemicals into the environment. By lysing competitors, allelopathy gives an undeniable advantage to A. minutum. While the mode of actions and effects have partially been elucidated at the cellular level, the effect of allelopathy at a community level remains to be investigated. Here, we investigated the "allelopathic shuttle": a new mechanism in which the allelopathic lysis results in the release of dissolved organic matter and an enhanced aggregation. The "allelopathic shuttle" could trigger a modification of the plankton community by inducing a sudden sink of competing microalgae, and favoring allelopathic cells. To test this hypothesis, the diatom C. muelleri was exposed to A. minutum cells or allelochemicals in coculture systems and roller-tanks (a culturing system allowing a constant sedimentation of plankton cells). Following the lysis of the diatoms, preliminary signs of aggregation were observed in the coculture system in presence of A. minutum. The formation of aggregates was confirmed within a few hours of coculture in the roller-tanks. Large sinking aggregates were formed in presence of dinoflagellate cells or filtrates. This study highlights for the first time the potential role of allelopathy in sedimentation and sinking of organic matter to the benthic compartment.

#### Modelling Primary Production at Lastovo Island (South Adriatic Sea)

### Matek, A.<sup>1</sup>, Ljubešić, Z.<sup>1</sup> & Kovač, Ž.<sup>2</sup>

<sup>1</sup>Department of Biology, Faculty of Science, University of Zagreb, Croatia <sup>2</sup>Department of Physics, Faculty of Science, University of Split, Croatia antonija.matek@biol.pmf.hr

#### THURSDAY 23<sup>rd</sup> August 2023 Room « Tali friz »

In vitro measurements are frequently used to recover the values of photosynthesis parameters, which are needed to model primary production. However, extraction of parameter values from *in situ* data was done relatively recently. In our study area around Lastovo Island, island internal trapped waves phenomena were observed and their effect on phytoplankton community during stratification was tested by measuring primary production *in situ*. Experiment was conducted in June 2022 at Maslovnjak (M1) and Struga (S0a) (opposite sides of Lastovo Island), and Prižba (P4) at Korčula Island (control station) using <sup>14</sup>C methodology. In situ normalized production profiles were measured and used to estimate photosynthesis parameters: assimilation number  $P^{B}_{m}$  and initial slope  $\alpha^{B}$ . Prior to analysis, a condition had to be met: normalized daily production declines with depth, which was observed in our data. The recovered parameter values were compared with distributions of  $P^{B}_{m}$  and  $\alpha^{B}$  estimated from *in situ* timeseries at Stončica station in the Adriatic. Model profiles of normalized daily production at Lastovo Island were computed and compared with *in situ* measured profiles to test model's efficiency of predicting primary production at each depth. If proved efficient, estimated parameters can be further utilized in numerical models of marine ecosystem and satellite remote sensing algorithms, or to extrapolate production values to non-measured depths.

### **KEYNOTE**

### The role of holopelagic *Sargassum* in the marine carbon cycle and its potential for carbon dioxide removal

Fernández-Méndez, M.<sup>1,2</sup>, Philippi, M.<sup>1</sup>, Thielecke, A.<sup>1</sup>, Schnetzer, J<sup>1</sup>., Behnke, J<sup>1</sup>., van Tussenbroek, B.<sup>2</sup>, Maga, E.<sup>2</sup>, Schwehm, N.<sup>3</sup> & Elmer, F.<sup>3</sup>

<sup>1</sup>HYIG Group Leader, Alfred Wegener Institute Helmholtz Center for Polar and Marine Research, Bremerhaven, Germany
<sup>2</sup>Unidad de Sistemas Arrecifales Puerto Morelos, Universidad Autonoma Nacional de Mexico, Mexico
<sup>3</sup>Seafields Solutions, UK
mar.fernandez-mendez@awi.de

Holopelagic sargassum (Sargassum fluitans and natans) is a floating brown macroalgae that originates from the Sargasso Sea and since 2011 creates extensive blooms across the tropical Atlantic. Despite its global relevance, this phenomenon is still poorly understood and can therefore not be represented in global Earth System Models. In this talk I will present results from four different field campaigns in Mexico and St Vincent performed between 2021 and 2023. We set up bioassays to quantify carbon and nutrient uptake rates at different nutrient concentrations, and remineralization rates of beached, processed and fresh sargassum. These were combined with in situ measurements of temperature, salinity, oxygen, pH, dissolved inorganic carbon, pCO<sub>2</sub>, particulate organic and inorganic carbon, and light reflection in natural and enclosed sargassum masses. Our results show that sargassums carbon uptake is decoupled from nutrient uptake, that processing sargassum slows remineralization, and that sargassum masses modify the biogeochemical properties of surface waters both within the patch and downstream enhancing carbon uptake. If sargassum is harvested before beaching, CO<sub>2</sub> emissions are avoided and the carbon sequestered in its biomass can either be stored for decades in long-lived products or it can be sunk to the deep sea for long-term sequestration. Our results will inform existing Earth System models to improve our understanding of the role of sargassum in the global carbon cycle and predict the potential of cultivating sargassum in open ocean aquafarms for additional large scale carbon dioxide removal.

#### ORALS

#### Brown algal fucoidan secretion and stability around the globe

Hellige, I.<sup>1,2</sup>, Buck-Wiese, H.<sup>1,2</sup>, Bligh, M.<sup>1,2</sup>, Arnosti, C.<sup>3</sup>, Ghobrial, S.<sup>3</sup>, Gustafsson, C.<sup>4</sup>, Kajee, M.<sup>5</sup>, Lloyd, C.<sup>3</sup>, Rothman, M.<sup>5</sup>, Salgado Murillo, B.<sup>7</sup>, Wieters, E.<sup>7</sup>, White, L.<sup>4</sup> & Hehemann, J-H.<sup>1,2</sup>

<sup>1</sup>University of Bremen, Faculty of Biology and Chemistry, Marum Centre for Environmental Research, Germany <sup>2</sup>Max-Planck Institute for Marine Microbiology, Germany <sup>3</sup>University of North Carolina at Chapel Hill, United States of America <sup>4</sup>University of Helsinki, Tvärminne Zoological Station, Finland <sup>5</sup>University of Cape Town, South Africa <sup>6</sup>Estación Costera de Investigaciones Marinas, Chile

ihellige@mpi-bremen.de

Brown algae invest significant quantities of fixed carbon dioxide into the secretion of complex polysaccharide fucoidan approaching the amount they store in biomass. The rates of fucoidan secretion and degradation by associated microbiomes remain unknown across different species of brown algae from different oceans. We quantify fucoidan secretion and degradation rates for *Lessonia trabeculata* in the South Pacific Ocean, *Ecklonia maxima* in the South Atlantic Ocean, *Sargassum fluitans* in the North Atlantic Ocean and *Fucus vesiculosus* in the Baltic Sea. The algae show high fucoidan secretion rates across the tested species. Preliminary experimental results show persistence against consumption of fucoidan by microbes during the initial degradation kinetic within weeks after secretion. The findings indicate fucoidan secretion is a general feature of brown algae. Initial persistence against consumption by bacteria are in line with fucoidan being a potential pathway for carbon sequestration around the globe.

# Mapping intertidal mudflat primary production by coupling hyperspectral remote sensing and CO<sub>2</sub> fluxes: from meso- to macroscale

Zhang, M.<sup>1</sup>, Polsenaere, P.<sup>2</sup>, Launeau, P.<sup>3</sup>, Jesus, B.<sup>1</sup>, Lavaud, J.<sup>4</sup>, Giraud, M.<sup>3</sup>, Rosa, P.<sup>1</sup>, Deborde, J.<sup>2</sup>, Geairon, P.<sup>2</sup>, Bonnefond, J-M.<sup>5</sup>, Lamaud, E.<sup>5</sup>, Grizon, J.<sup>2</sup> & Méléder, V.<sup>1</sup>

<sup>1</sup>Nantes Université, Institut des Substances et des Organismes de la Mer, ISOMer, BP 92208, 44322 Nantes, France

<sup>2</sup>*IFREMER, Laboratoire Environnement et Ressources des Pertuis Charentais (LER-PC), BP133, 17390, La Tremblade, France.* 

<sup>3</sup>Nantes Université, LPGN, UMR CNRS-6112, BP 92208, 44322 Nantes, France

<sup>4</sup>UMR6539 LEMAR-Laboratoire des Sciences de l'Environnement Marin, Institut Européen de la Mer, Technopôle Brest-Iroise, rue Dumont d'Urville, 29280 Plouzané, France

<sup>5</sup>ISPA UMR 1391 – INRA, CS 20032, 33882 Villenave d'Ornon cedex, France

meng.zhang777777@gmail.com

Intertidal mudflats, present all over the world are excluded from global carbon budget calculation; while these ecosystems are increasingly recognized to be as productive as tropical forests. With an annual

#### THURSDAY 23<sup>rd</sup> August 2023 Room « Tali friz »

global productivity estimated to be in the order of 500 million tons of carbon, mudflats can therefore represent up to 20% of the global production of the oceans, whereas they occupy only 0.03% of their surface, with a total area estimated at 130,000 km<sup>2</sup>. Despite their potentially high contribution to the overall carbon budget, their actual contribution remains unknown. Moreover, these ecosystems are currently under threat from global climate changes and increased human activities. In this context, estimating the actual carbon uptake by these ecosystems from the local to the global scale is a challenge that has to be tackled, which is the objective of this project.

The main innovation of our project resides in coupling hyperspectral remote sensing, with  $CO_2$  fluxes measured at different spatial-temporal scales using benthic chambers and atmospheric eddy covariance. The final objective is to map the gross primary production (GPP) to estimate for the first time the contribution of these ecosystems at the global carbon cycle. This tool will help predicting what will happen to the tidal ecosystems due to changes linked to global climate changes and assist in developing mitigation and adaptation strategies to comply with the objectives of the Paris Agreement.

### SYMPOSIUM 20 "Coastal and freshwater systems under human pressure: status assessment, management and conservation"

#### KEYNOTE

# Assessing ecological water quality of Mediterranean lakes/reservoirs using phytoplankton indices

Katsiapi, M.<sup>1,2</sup>, Stefanidou, N.<sup>2</sup>, Alexiadou, D.<sup>1</sup>, Xanthopoulou, N.<sup>1</sup>, Christodoulou, A.<sup>1</sup>, Lioumbas, I.<sup>1</sup>, Stamou, G.<sup>2</sup>, Polykarpou, P.<sup>3</sup>, Dörflinger, G.<sup>3</sup>, Michaloudi, E.<sup>2</sup> & Moustaka, M.<sup>2</sup>

<sup>1</sup>EYATH SA (Thessaloniki Water Supply & Sewerage Company), Thessaloniki, Greece <sup>2</sup>School of Biology, Aristotle University of Thessaloniki, Thessaloniki, Greece <sup>3</sup>Water Development Department, Pallouriotissa, Nicosia, Cyprus mkatsiapi@eyath.gr

Freshwaters worldwide are in high risk of anthropogenic modifications leading to increased eutrophication phenomena and water quality degradation. For ecological water quality classification, indices characterizing the status of biological elements have been developed during the Water Framework Directive (WFD) implementation. Among biological elements for water quality assessment, phytoplankton is of particular interest due to its direct response to nutrient enrichment and/or physical disturbances through changes in biomass and composition. A high number of phytoplankton indices have been developed in Europe including ones used in the Mediterranean lakes and reservoirs. In this work we review phytoplankton indices used during the WFD implementation in Greece and other Mediterranean countries to classify ecological water quality. Our primary goal is to focus on comparability of ecological water quality assessment across Europe by examining ecological standards transcending geographic regions and national boundaries behind phytoplankton metrics and indices. Paradigms are presented from Greek natural lakes and reservoirs and reservoirs in other Mediterranean countries.

### ORALS

# How the ongoing oligotrophication of the northern Adriatic affects phytoplankton community structure and functioning

Mozetič, P.<sup>1</sup>, Petelin, B.<sup>1</sup>, Flander-Putrle, V.<sup>1</sup>, Slavinec, P.<sup>1,2</sup>, Vascotto, I.<sup>1,3</sup> & Francé, J.<sup>1</sup>

<sup>1</sup>National Institute of Biology, Marine Biology Station Piran, Slovenia <sup>2</sup>University of Ljubljana, Biotechnical Faculty, Slovenia <sup>3</sup>Jozef Stefan International Postgraduate School, Ljubljana, Slovenia Patricija.mozetic@nib.si

#### THURSDAY 23<sup>rd</sup> August 2023 Room « Tali friz »

Predicting the effects of climate change and other anthropogenic stressors on plankton communities remains challenging due to the complex interactions between environmental factors and plankton. In the coastal ecosystems of the northern Adriatic Sea, climate-induced changes combined with human activities in the catchment resulted in an imbalanced nutrient status in the seawater, leading to a previously reported basin-wide decrease in trophic status in the early 2000s that coincided with the globally observed decline in phytoplankton biomass.

To investigate whether phytoplankton biomass has subsequently recovered and how this is reflected in phytoplankton community structure and functioning, we analyzed a 30-year time series (1989-2021) of phytoplankton and environmental data from a coastal LTER site in the Gulf of Trieste (northern Adriatic). The most striking feature is the increase in seawater temperature at all depths (0.03 °C year<sup>-1</sup>), especially in the last decade. Surface salinity fluctuates on multi-year to multidecadal scale, following the alternation of floods and droughts in rivers, which are the main source of new nutrients. In an already P-limited ecosystem, the steady decline in dissolved inorganic nitrogen concentrations from 2015, coinciding with the onset of a "dry" period, has resulted in phytoplankton biomass (as Chl-a concentrations) being even lower than in the early 2000s.

The phytoplankton community has adapted to these oligotrophic conditions through changes in size related functional traits and phenology. The increasing irregularity of the annual phytoplankton cycle toward the end of the time series reduces the predictability of phytoplankton phenology and of ecosystem functioning.

## Organic Contaminants fate in aquatic ecosystems, which role for microalgae?

Cheloni, G.<sup>1</sup>, Félix, C.<sup>1</sup>, Le Floc'h, E.<sup>1</sup>, Rosain, D.<sup>2</sup>, Courant, F.<sup>2</sup>, Gomez, E.<sup>2</sup>, Leboulanger, C.<sup>1</sup> & Fouilland, E.<sup>1</sup>

<sup>1</sup>UMR MARBEC, Univ. Montpellier, CNRS, IFREMER, IRD, Sète, France <sup>2</sup>HydroSciences Montpellier, IRD, CNRS, University of Montpellier, Montpellier 34093, France giulia.cheloni@cnrs.fr

Microalgae are reference organisms for toxicity testing and risk assessment of organic contaminants (OCs) in aquatic ecosystems. Extensive information is now available on the toxic effects of OCs on microalgal physiology. On the other hand, investigations on the role of microalgae on OCs fate were neglected though required to assess contaminants persistence and trophic transfer, to identify biomarkers of contaminants exposure and to improve microalgae-based water remediation strategies. Genome sequencing of model microalgal species revealed the presence of genes encoding for enzymes potentially involved in OC biotransformation. However, knowledge about biotransformation pathways in microalgae lag far behind that of other microorganisms.

In our study *Phaeodactylum tricornutum* was exposed to three OCs, two pharmaceuticals (Diclofenac, Ethynilestradiol) and the herbicide Diuron. OC effects on cellular traits and physiology were studied together with OC removal from the exposure medium (HPLC-UV). The three contaminants presented different modes of toxic action. Diuron inhibited cell growth (EC50=10µgL<sup>-1</sup>) and photosynthetic activity, Diclofenac affected cell survival ([Diclofenac]>10mgL<sup>-1</sup>) but not through alteration of photosynthetic activity nor generation of oxidative stress. Finally, Ethynilestradiol induced oxidative stress ([Ethynilestradiol]>4mgL<sup>-1</sup>) and altered cell morphology with lipid droplets formation. Significant removal after 72h incubation was observed only for Ethyniestradiol (up to 60% for the tested concentrations). Obtained results confirm that phytoplankton may influence the fate of contaminants in the water column. However, this process changes depending on the OC. Ongoing analyses for

contaminants transformation (HPLC-MS) and transcriptome (RNA-seq) responses will shed light on the activated metabolic pathways possibly involved in the transformation processes.

# Using niche analysis as a steppingstone towards a new diatombased biotic index in transitional waters

Ribeiro, L.<sup>1</sup>, Hernandez-Fariñas, T.<sup>2</sup>, Oiry, S.<sup>3</sup>, Du, G.<sup>4</sup>, Brotas, V.<sup>1</sup> & Barillé, L.<sup>3</sup>

<sup>1</sup>MARE – Marine and Environmental Sciences Centre, Portugal <sup>2</sup>IFREMER, France <sup>3</sup>ISOMER, France <sup>4</sup>Key Laboratory of Marine Genetics and Breeding, China Ilribeiro@fc.ul.pt

Coastal and transitional waters host microphytobenthos (MPB), dominated by diatoms, which greatly contribute to sediment stabilization, carbon transfer, and up to half of estuarine primary production. However, its potential as a biological indicator is yet to be explored. Diatom-based metrics have been successful in detecting eutrophication or organic pollution in freshwater lentic and lotic systems but transposing them directly to mudflat assemblages is of limited applicability due to insufficient auto-ecological data and natural estuarine dynamics.

To address this challenge, a niche analysis approach was used in this study. Ecological niches of 160 benthic diatom species were analyzed using Outlying Mean Index ordination method, incorporating biomass-weighted species abundances and relevant environmental variables from datasets collected in mudflats of the polyhaline sectors of three European estuaries (Aiguillon Bay, Loire, and Tagus). Tolerance (i.e., niche breadth) as well as niche position values are dependent on both each taxon's relative abundance and biomass values, as well as the subset of environmental variables available. The information provided by the main niche parameters and is discussed regarding their significance for bio-indication, namely as proxies to indicator (niche breadth) and sensitivity (niche position) values used in indexes based on the Zelinka and Marvan formula.

# Biogeochemical modelling of eutrophication in coastal waters. An ensemble model approach for the calculation of threshold levels

Plus, M. & OSPAR ICG-EMO

*Ifremer, Dyneco Pelagos Laboratory, Brest, France. \*martin.plus@ifremer.fr* 

Despite the general understanding of the drivers and the processes leading to eutrophication, there is still a need to improve the assessment of the eutrophication status of coastal waters. European legilations (Water Framework directive, WFD, Marine Strategy Framework Directive, MSFD) impose the member states to recover a Good Ecological/Environmental Status (GES) in all European water bodies by 2027. In order to make progress toward consistent threshold values indicating the GES, biogeochemical models are valuable tools that provide quantified targets for improving the health of coastal ecosystems. Within the framework of a ensemble modelling exercise lead by the OSPAR Intersessional Correspondance Group on Eutrophication Modelling, several 3D models coupling ecology and hydrodynamics, were used to simulate the pre-eutrophic conditions ('historic' simulation, HS) that prevailed in 1900, before the industrialization and agricultural intensification, as well as the 'current state' conditions (CS, 2009-2014). A weighting method based on comparisons between CS and

observations, allowed to propose threshold levels for DIN, DIP and chlorophyll a, taking into account regional differences in model performances. The ECOMARS-3D model, is one of such biogeochemical models, it has been applied to an area covering the Bay of Biscay, the Celtic Sea and the English Channel. By presenting this model results and its ability to simulate CS observations, as well as the different modelling steps that took place in this exercise, we will describe the processes that allowed to propose GES threshold levels for the OSPAR assessment areas.

### **KEYNOTE**

# Macroalgal monitoring challenges under the climate-driven community changes

Ramos, E., Guinda, X., de la Hoz, C.F., Puente, A. & Juanes, J.A.

IHCantabria - Instituto de Hidráulica Ambiental de la Universidad de Cantabria, Spain ramose@unican.es

Marine classification is a useful predictive tool for a variety of assessment purposes, which facilitate the quantification of responses in biological patterns and processes to human uses at a certain region. It may also be useful for the development of conservation strategies to preserve species in degraded or fragmented areas, as well as in habitats shifting due to climate change.

In the context of the WFD, it was necessary to perform the intercalibration of different methodologies for the assessment of the biological quality element vegetation. The NEA coast presents such a diverse macroalgae composition that it was difficult to establish common standardized assessment methods and reference conditions within the whole area. The recognition of "common suitable types" was then an urgent need and a preliminary task before intercalibration can be finalized. Taking into account this necessity, a classification based on physical variables was developed, which was validated with intertidal macroalgae data. This classification can be used on areas of higher affinities, establishing groups inside which communities can be compared and intercalibration performed in a more precise way.

Despite the importance of biogeographic approaches, classifications at a finer resolution level may be useful for conservation planning and for the implementation of effective biomonitoring programs in a particular region.

Nowadays, given the important impact of climate change on macroalgae distribution, these classifications based on physico-chemical data may be projected in order to analyse the vulnerability of different areas under climate change scenarios.

#### ORALS

#### Cultivating sugar kelp as an instrument for mitigating eutrophication

Bruhn, A.<sup>1</sup>, Boderskov, T.<sup>1</sup>, Christiansen, F.<sup>1</sup>, Holding, J.<sup>1</sup>, Holst, N.<sup>2</sup>, Høgslund, S.<sup>1</sup>, Mostovaya, A.<sup>1</sup>, Nielsen, M.M.<sup>3</sup>, Pedersen, S.G.<sup>1</sup>, Schmedes, P.S.<sup>3</sup>, Taylor, D.<sup>3</sup>, Vinbæk, S.J.<sup>1</sup> & Timmermann, K.<sup>3</sup>

<sup>1</sup>Aarhus University, Department of Ecoscience, Denmark
 <sup>2</sup>Aarhus University, Department of Agroecology, Denmark
 <sup>3</sup>Danish Technical University, Department of Aquatic Resources, Denmark

anbr@ecos.au.dk

As all EU countries, Denmark is obliged in the EU Water Framework Directive to achieve 'good ecological conditions' in marine coastal waters before 2027. In most coastal areas, this will require instruments to be taken into consideration for mitigating eutrophication, in addition to the presently decided reduction of the nutrient emissions from land. Large-scale cultivation of sugar kelp (*Saccharina latissima*) is one of the instruments considered by the Danish Environmental Authorities.

In an ambitious national project, the potential of cultivation of sugar kelp for mitigation of eutrophication has been investigated through a large-scale cultivation effort: A 12-hectare seaweed test farm was established and run from 2022 to 2023. Harvest in late April 2023 documents record high harvest yields for Danish conditions with average biomass yields of up to 7 kg fresh weight per linear meter of cultivation line.

Environmental impacts on hydrological conditions, carbon, nutrient and oxygen dynamics in the adjacent water column and sediment, as well as climate gas emissions were investigated through environmental and laboratory campaigns before, during and after cultivation. Cost efficiency of cultivating sugar kelp for mitigating eutrophication was estimated, testing commercial applications of the harvested seaweed for animal feed and food ingredients. Model tools were developed to predict the mitigation potential of the sugar kelp production in all specific Danish coastal areas.

Results will be discussed in the perspectives of the potentials and pitfalls of future coastal management supporting at the same time marine nature restoration and the circular blue bioeconomy.

### Photosynthetic and growth responses of *Ulva lacinulata* to survive the fluctuating conditions in eutrophic Thessaloniki Bay, Greece

Orfanidis, S., Malea, L., Nakou, K., Seitidou, O., Lachanidou, G. & Papadimitriou, A.

*Fisheries Research Institute (ELGO-DIMITRA), 640 07 Nea Peramos, Kavala, Greece* sorfanid@inale.gr

*Ulva lacinulata* (Kützing) Wittrock was sampled nine times from the eutrophic Thessaloniki Bay, Greece, between June 2019 and March 2021 to study photosynthetic and growth responses: (a) to explain species' seasonal acclimation strategies and (b) to support species' sea or land-based mass cultivation. Critical seawater variables were analysed by using oneway ANOVA and were correlated with subcellular (Rapid Light Curve, JIP-test, Photochemical Quenching analysis, and pigment content) and organismal (Relative Growth Rate-RGR) species variables that were investigated under field conditions simulated in the laboratory by using multivariate analysis (RDA).

The results showed a statistically significant variation (p<0.05) for the factor time of all (26) tested physiological parameters. RDA analysis showed temperature as the main factor (p<0.001) affecting the species' photosynthetic performance (qP,  $\Phi$ PSII, rETRmax, Ek), which was higher during the warm and lowest during the cold period. However, the species' acclimation capacity to low was higher than to high temperatures. Indeed, increased Chlorophyll/Carotenoids ratios at 27-28°C in July and September 2019, except in July 2020 (after Covid-19 lockdown), indicated stress or damage to the photosynthetic apparatus. A heavy rainfall on June 2019 that lowered the salinity and accumulated nitrogen, and suspended sediment reduced the photosynthetic efficiency (alpha) and the density of active Reactive Centres per Photosystem II (RC/ABS).

#### THURSDAY 23<sup>rd</sup> August 2023 Room « Tali friz »

Maximum RGR values that were estimated during March 2021 coincided with the maximum biomass in the field. Based on the species' potential monthly growth yield (>70% of the maximum), the farming at the collection site could be successful from October to June.

### SYMPOSIUM 16 "Advances in algal production processes"

#### **KEYNOTE**

### Improving the sustainability of algae production in commercial scale facilities

Pereira, H.<sup>1</sup>, Rodrigues, A.<sup>1,2</sup>, Cardoso, H.<sup>1,3</sup>, Varela, J.<sup>1,4</sup> & Navalho J.<sup>1,2</sup>

<sup>1</sup>GreenCoLab – Associação Oceano Verde, University of Algarve, Campus de Gambelas, 8005-139 Faro, Portugal

<sup>2</sup>Necton S.A., Belamandil, 8700-152 Olhão, Algarve, Portugal

<sup>3</sup>Allmicroalgae Natural Products S.A., Pataias, Portugal

<sup>4</sup>Centre of Marine Sciences, University of Algarve, Campus de Gambelas, 8005-139 Faro, Portugal.

hugopereira@greencolab.com

Algae are among the most promising feedstocks with a growing demand, aiming to make a real, tangible and positive difference for a greener and healthier Europe. Although the algae sector is still an emerging industry, there is a growing commercial interest from different industrial and commercial stakeholders in using algae biomass as a more sustainable feedstock. Nevertheless, the commercial production of algal biomass still displays relatively high costs, mainly related to the high energy consumption and human labour associated with large-scale production processes. Therefore, a significant R&D effort to develop sustainable production processes to substantially reduce production costs is key to allowing the industry to thrive and harness the effective introduction of algal feedstocks in different applications and novel value chains. In this context, the presentation will address different production strategies conducted at industrial-scale facilities to improve the overall sustainability of the production processes, aiming to reduce production costs significantly. The optimisation includes strategic actions in line with the circular economy principles that rely on integrating production inputs (water, nutrients and CO<sub>2</sub>) from adjacent industries in large-scale facilities. Additionally, specific strategies to improve biomass productivity in industrial settings as well as sustainable algae cultivation and processing (e.g., solar drying) approaches will be discussed. The ultimate goal of the combined optimisation strategies is to increase the overall sustainability of the production processes to unlock the full potential of algae feedstocks for different biotechnological applications.

### ORALS

#### Strain selection in Ulva spp. for land-based aquaculture

Nehr, Z.<sup>1</sup>, Potin, D.<sup>1</sup>, Jouanneau, D.<sup>1</sup>, Jacob J.F.<sup>2</sup>, & Potin, P.<sup>1</sup>

<sup>1</sup>UMR8227 Integrated Biology of Marine Models, Station Biologique de Roscoff, France <sup>2</sup>Bezhin Breizh / WeaSea, France znehr@sb-roscoff.fr

As fast-growing species in land-based aquaculture systems, *Ulva* spp attract great interest as a source of edible seaweed and high value bioactive products that can be valorized in cosmetics, nutraceutics and pharmacology. A strain selection program was designed with the aim to create a locally sourced

collection of *Ulva spp.* strains among which best fitting strains will be picked and cultivated for specific downstream applications. 300 wild strains were collected around Brittany and kept in culture in the lab. This collection covers the five species that can be found along the Breton coast among which, 123 did not display any reproduction. To identify best performing strains, we evaluate growth rate on a custom made platform. The 30 best growing strains had a growth rate ranking from 5 % to 30 % a day. To optimize the yield, they were then grown in 19 different nutrient conditions and phenotyped for biochemical content. Various traits were measured : growth rate, dry matter content, cell wall polysaccharide content (ulvan), protein content, pigment and total nitrogen. This work will result in a subset of strains, each one exhibiting an optimized yield in one of the molecules of interest. The best performing strains will be transferred in farming conditions and serve as parental lines for future selective breeding.

# Priming as a kelp crop-enhancement strategy for seaweed cultivation under environmental challenges

Gauci, C.<sup>1,2</sup>, Hill, G.<sup>1</sup>, Hoarau, G.<sup>1</sup>, Jueterbock, A.<sup>1</sup> & Bartsch, I.<sup>2</sup>

<sup>1</sup>Faculty of Life Sciences and Aquaculture, Nord University, Norway <sup>2</sup>Alfred-Wegener-Institute, Helmholtz Centre for Polar and Marine Research, Germany clement.gauci@nord.no

Wild harvesting of kelps for commercial purposes puts considerable pressure on these habitats. It is thus of importance to support kelp aquaculture and develop crop enhancement techniques. Priming, a plant's ability to acquire a stress memory via epigenetic alteration, is a promising feature to cope with climate change in higher plants.

We used this concept and investigated the potential of temperature priming to improve growth and survival of Saccharina latissima, the most cultivated kelp species in Europe. Because it is unknown, how long a priming signal has to act to trigger an effect in kelps, we exposed kelp gametophytes to a sublethal temperature of 20°C for 0, 2, 4 and 6 weeks. After priming of gametophytes, the speed of gametogenesis and growth of juvenile sporophytes was followed at 5°C for 3 weeks. In a second step, the resulting sporophytes were transferred to a heat stress treatment at 20, 22, 23 or 24°C over two weeks to quantify sporophyte survival.

We hypothesized successfully that primed strains exhibited a higher growth performance and a higher thermal tolerance compared to non-primed strains. A priming time of 2 weeks was optimal: gametophytes exhibited fastest gametogenesis and formed most sporophytes at 5°C. However, the most temperature resistant sporophytes, with 1°C higher tolerance than controls, developed from gametophytes primed for 4 weeks.

These results suggest that priming is a promising technique for kelp cultivation under warmer climates as well as for restoring natural kelp forests affected by heat waves, but would likely require different priming strategies.

### KEYNOTE

#### Green Chemistry through Photosynthetic Microbial Biocatalysts

Allahverdiyeva, Y.

Molecular Plant Biology, Department of Life Technologies, University of Turku, Finland \*allahve@utu.fi

Photosynthetic microorganisms, such as microalgae and cyanobacteria, have immense potential for carbon-neutral production of desired compounds and can play a crucial role in sustainable blue bioeconomies. To enhance bioproduction efficiency, we are exploring the regulation of photosynthesis and engineering electron transport pathways to direct photosynthetic reducing power towards targeted chemicals.

In a 'direct solar fuels' production scenario, we designed a pulse-illumination protocol to prevent the activation of competitive electron sinks and accumulation of  $O_2$ , thereby enhancing photo-hydrogen production in *Chlamydomonas*. Furthermore, the removal of the Mehler-like reaction catalyzed by flavodiiron proteins significantly improved the channelling of photosynthetic electrons towards hydrogenase in *Chlamydomonas*.

In a 'photobiotransformation' scenario, we integrated heterologous oxidoreductases into cyanobacteria or green algae to use photosynthetic reductants (NADPH) for converting externally added substrates into high-value compounds. By wiring recombinant enzymes as a strong electron sink to the electron transfer chain, we fully exploit photosynthetic light reactions while outcompeting alternative electron transport routes.

Finally, in a 'solid-state production platform', we 3D-print photosynthetic cell factories in a leaf-inspired hierarchical architecture of bio-based polymers that allows us to control energy loss to biomass, transport of products and gases, as well as nutrient fluxes. Overall, the use of photosynthetic microorganisms as cell factories has the potential to revolutionize biomanufacturing and contribute to sustainable development.

#### ORALS

# Bio-inspired materials for increasing the polyunsaturated fatty acids productivity of microalgae: *Lobosphaera* immobilized on polyethylenemine and chitosan-based carriers

Vasilieva, S.G.<sup>1,2</sup>, Lobakova, E.S.<sup>1</sup>, Shibzukhova, K.A.<sup>1</sup>, Grigoriev, T.E.<sup>3</sup>, Antipova, C.G.<sup>3</sup>, Chivkunova, O.B.<sup>1</sup>, Lukyanov, A.A.<sup>1</sup> & Solovchenko, A.E.<sup>1,2</sup>

<sup>1</sup>Faculty of Biology, Lomonosov Moscow State University, Russia
 <sup>2</sup>Institute of Natural Sciences, Derzhavin Tambov State University, Russia
 <sup>3</sup>Laboratory of polymeric materials, National Research Center "Kurchatov Institute", Russia vankat2009@mail.ru

Immobilization of microalgae cells (MA) facilitates their harvesting constituting a major challenge in algal biotechnology. Increased cell density, lower risk of contamination, and higher resistance to various stresses can enhance immobilized MA productivity regarding valuable metabolites as compared with the corresponding suspended cultures.

The chlorophyte strain *Lobosphaera* sp. IPPAS C-2047, which is the prospective candidate for the efficient phototrophic production of arachidonic, linoleic and  $\alpha$ -linolenic acids, served as the object in the present work. The solid, highly porous and hydrophilic polyethylenemine (PEI) and chitosan-based polymers (CH), synthesized by method of cryopolymerization with diethylene glycol diglycidyl ether and glutaraldehyde, were tested as the MA carriers.

The profound difference of the effect of immobilization on PEI and CH on lipid metabolism of *Lobosphaera* sp. was revealed. The immobilization on PEI increased ARA percentage in the total tatty acids and ARA volumetric productivity by 72% and 81%, compared with the suspended cells cultured

#### THURSDAY 24<sup>th</sup> August 2023 Room « Tali melen »

respectively in complete or nitrogen-deprived medium 14 days. Immobilization on CH declined ARA but increased oleic and  $\alpha$ -linoleic acid percentages. Importantly, total volumetric FA content in the cells immobilized on both carrier types was 15–35% higher than that of the suspended cultures regardless of nitrogen availability.

In view of the advantages of attached cultivation, immobilization of *Lobosphaera* sp. IPPAS C-2047 on the CH- and PEI-based polymeric cell carriers is a promising avenue for the development of viable technology for increasing of polyunsaturated fatty acids productivity yield and especially, productivity of *Lobosphaera* towards the arachidonic, linoleic and  $\alpha$ -linolenic acids.

#### Effect of an optical lightguide on growth of Arthrospira platensis

Sasi, A.<sup>1</sup>, Turnbull, G.A.<sup>2</sup>, Edwards, C.<sup>2</sup>, Lawton, L.<sup>2</sup> & McKenzie, D.<sup>3</sup>

<sup>1</sup>University of St Andrews, United Kingdom <sup>2</sup>Robert Gordon University, United Kingdom <sup>3</sup>Xanthella Ltd., United Kingdom as606@st-andrews.ac.uk

Microalgae can help in capturing carbon from multiple sources and large-scale photobioreactors will play major role in achieving carbon neutrality. Illumination is one of the major challenges in high cell density algae cultures and the current systems used in photobioreactors can be costly, bulky and inefficient. A robust lighting system has been developed that improves the light penetration into the bulk of the photobioreactor. The modular design of the light source also makes it easy to maintain and repair. The illumination setup comprises of a single white LED emitter (Cree XHP50) optically coupled to an acrylic light guide. The surface of the light guide has perforations to distribute the light evenly across its length. Optical simulation software was used in designing the optimal shape and structure of the light guide for achieving uniform light distribution throughout the depth of the photobioreactor. Initial growth studies were performed in a cylindrical air-lift photobioreactor to evaluate the efficiency of the light guide. Spirulina grown using the lightguide had a growth rate of 0.128 d<sup>-1</sup> compared to 0.072 d<sup>-1</sup> for previous studies cultivated using a single white LED without light guide. The novel light source could also illuminate 3700 cm<sup>3</sup> of the 10 L photobioreactor. We will also present a growth comparison study on Spirulina evaluating the effect of the lighting system on cell density, growth rate and pigment production. The work will also discuss the impact of shading effects, scattering and absorption of light by microalgae cells at high cell densities.

# SYMPOSIUM 14 "Algal culture collections: their increasing societal relevance and role as gene repositories"

#### **KEYNOTE**

# Microalgal culture collections: their roles and challenges in the $\mathbf{21}^{th}$ century

Chaerle, P.<sup>1</sup> & Wilmotte, A.<sup>2</sup>

<sup>1</sup>BCCM/DCG collection of diatoms, PAE, Ghent University, Belgiun <sup>2</sup>BCCM/ULC collection of cyanobacteria, InBios, University of Liège, Belgium awilmotte@uliege.be

Culture collections of cyanobacteria and eukaryotic microalgae play major roles in important processes: 1) Biodiversity conservation and long-term safeguarding; 2) Expanding the limits to our knowledge of biology; 3) Use of biological resources for the benefit of society.

In an ever-evolving context, they also have to deal with challenges related to these roles. Some challenges are technical, like the improvement of isolation and purification methods, of preservation protocols, and taxonomic characterization tools. They may also broaden their scope to the preservation of whole communities instead of isolates. Other challenges are more legal and bureaucratic, with the implementation of the Budapest Treaty for patent strains, and the Nagoya Protocol for all strains. Most culture collections are subsidized by public national structures, but are asked to generate revenues. This might originate from provided services and trainings, but a more beneficial valorization of their patrimony and expertise might come from collaborations with industry funders. However, this latter requests an extra layer of legal and business development support.

The recently started European Research Infrastructure "Microbial Resource Research Infrastructure" (MIRRI) aims to gather interested culture collections of all kinds of microorganisms and genetic material around a central access platform (https://www.mirri.org/).

Thus, the present landscape of the culture collections of microalgae is now stretched from the regional to the European scale.

We think that it is important to preserve the variety of culture collections, small and large, reflecting the huge diversity of the organisms they hold and the complexity of skills all of them possess.

### ORALS

# Cryopreservation of diverse Symbiodiniaceae species: fatty acid profiles, growth rates and photosynthetic performance

Kihika, J.<sup>1,2</sup>, Wood, S.<sup>1</sup>, Rhodes, L.<sup>1</sup>, Smith, K.<sup>1,3</sup>, Miller, M.<sup>1</sup>, Pochon, X.<sup>1,4</sup>, Thompson, L.<sup>1</sup>, Butler, J.<sup>1</sup>, Oakley, C.<sup>2</sup> & Ryan, K.<sup>2</sup>

<sup>1</sup>Cawthron Institute, New Zealand

<sup>2</sup>Department of Biological Sciences, Victoria University of Wellington, New Zealand

<sup>3</sup>Department of Biological Sciences, University of Auckland, New Zealand <sup>4</sup> Marine Science Institute, University of Auckland, New Zealand joseph.kihika@vuw.ac.nz Symbiodiniaceae are a diverse group of dinoflagellates, which can be both free-living and/or associated with a variety of protists and other invertebrate hosts. Maintenance of isolated strains in culture is labourintensive and expensive. Cryopreservation provides an excellent avenue for their long-term storage. Different cultured isolates from six Symbiodiniaceae genera were cryopreserved using either rapid freezing or controlled rate freezing techniques at both 10% and 15% of dimethyl sulfoxide (DMSO) as the cryoprotectant agent (CPA). For the isolates that had low survival rates during cryopreservation, increased salinity treatments were used before freezing.

There were high cell viabilities in *Symbiodinium pilosum* cultures after treatment with increased salinities of 44 parts per thousand (ppt) and 54 ppt culture medium and *Fugacium* sp. after salinity treatments of 54 ppt and 64 ppt. Fatty acids profiles changed in response to increased salinity treatments as compared to normal salinity (34 ppt). Assessment of the photosynthetic performance of both endosymbiotic and free living Symbiodiniaceae isolates using Pulse Amplitude Modulated (PAM) fluorometry showed that cryopreservation did not affect their photosynthetic ability nor growth rates. Molecular investigations are underway to explore whether genes involved in enhancing cryopreservation success can be identified.

#### Algal culture collections in a changing world

#### Lorenz, M.

Experimental Phycology and Culture Collection of Algae (SAG), Albrecht-von-Haller Institute for Plant Science, Georg-August-University Goettingen, 37073 Goettingen, Germany mlorenz@uni-goettingen.de

Algae and cyanobacteria play a major role in our world's geochemical and biological cycles. Their high phylogenetic diversity is reflected in a multitude of physiological pathways and survival strategies, that bear high potential to meet current societal challenges and support bioeconomy. Beside strong scientific foundations, all this requires improved availability and quality of phycological resources and metadata. The Culture Collection of Algae at Goettingen University (SAG) is a large algal service culture collection and modern biological resource centre (BRC) with more than 2300 strains in its open collection.

For seventy years now, SAG provides high quality reference material and metadata and adds its specific expertise to many fields of fundamental and applied phycology. Current examples include research on whole genome sequencing, functional genomics, ecotoxicology and biophysics. SAG's resources and expertise underpin species identification projects using e.g. metabarcoding or Artificial Intelligence (AI) technology for biodiversity research and protection.

The quantity and quality of metadata connected to the holdings of algal culture collections (ACCs) rapidly increases, while the quality-enhancement (e.g. axenization) and expansion of diversity and number of holdings seems to be more restricted to limited resources and methods. To overcome these restrictions ACCs play a crucial role in generating and developing long-term maintenance methods of genetically stable and easily accessible algal resources.

### KEYNOTE

Vaulot, D.

### SYMPOSIUM 08 "Algal photosynthesis, carbon fixation and respiration"

### **KEYNOTE**

# Measurements of photosynthetic activity in algal mixtures reveal biotic interactions between diatoms and dinoflagellates

Peltekis, A.<sup>1</sup>, Gain, G.<sup>1</sup>, Mével, G.<sup>1</sup>, Croteau, D.<sup>1</sup>, Ferté, S.<sup>1</sup>, Israelievitch, E.<sup>1</sup>, Guillou, L.<sup>2</sup>, Fontana, A.<sup>3</sup> & Bailleul, B.<sup>1</sup>

<sup>1</sup>UMR7141, Institut de Bilogie Physico-Chimique, CNRS/Sorbonne Université, Paris, France <sup>2</sup>UMR7144, Station Biologique de Roscoff, Sorbonne Université, France. <sup>3</sup>Institute of Biomolecular Chemistry, CNR, University of Naples, Italy

In the ocean, the dynamics and structure of phytoplankton assemblages depend on many factors including light irradiance, turbulence, nutrient availability, but also allelopathy, i.e. the release of allelochemicals harmful to the physiology of competitors. We have developed a new approach to measure the photosynthetic activity of each species within a mixture, based on the electrochromic shift (ECS) of photosynthetic pigments, which displays a different spectral signature in each photosynthetic clade of microalgae.

This method revealed that the photosynthetic activity of several diatoms, including *Thalassiosira pseudonana*, was inhibited when mixed with the dinoflagellate *Amphidinium carterae*. This inhibition is caused by the excretion by the dinoflagellate of a secondary metabolite of the amphidinol family, which suppresses the proton motive force in the thylakoids of the diatom. Using diatom photosynthesis as a proxy, we investigated abiotic factors (turbulence, nutrients, light) influencing amphidinol production and excretion. During the growth of *A. carterae*, we identified three stages: (i) during the exponential phase, amphidinols are not produced; (ii) as soon as the stationary phase is reached, the concentration of intracellular amphidinols increases very suddenly, (iii) later in the stationary phase, large quantities of amphidinols are secreted. The production of amphidinols is further increased under conditions of phosphate starvation and turbulence increases their excretion.

This new methodology allows for a screening of allelopathic interactions within phytoplankton and reveals the potential of photosynthesis as a tool for stress diagnostics and environmental monitoring in the ocean.

### ORALS

# Comparative analysis of photosynthetic adaptive strategies to Fe limitation in the marine diatoms *Thalassiosira pseudonana* and *Thalassiosira oceanica*

Angulo, J., Finazzi, G., Courtois, F., Curien, G. & Menneteau, M.

Laboratoire de Physiology Cellulaire et Végétale (LPCV), Univ. Grenoble Alpes, CNRS, CEA, INRAE, Grenoble, France

jhoanell.angulo@cea.fr

Coastal and oceanic marine environments differ in several aspects, including e.g. their bioavailable metal content (Fe, Mn, Zn). Oceanic phytoplankton species must lower their metals requirements for growth, to thrive in oligotrophic environment (Sunda et Huntsman 1995; Peers et Price 2006). Previous studies have reported tolerance to low-iron conditions in the open ocean diatom Thalassiosira oceanica when compared to related coastal species, including e.g. Thalassiosira pseudonana. The lower iron requirement in T. oceanica has been partly explained by the replacement of photosynthetic iron-rich proteins by enzymes containing other metals (Peers et Price 2006; Strzepek et Harrison 2004). However, the effect of this remodeling on the cell bioenergetics and organelle cross talk is not entirely known. In this work, we use absorption spectroscopy, in-vivo chlorophyll fluorescence and oxygen measurements to compare the effect of iron deficiency on photosynthesis in both oceanic and coastal Thalassiosira species. Preliminary results obtained during the first year of my PhD indicate that iron-limited condition induces a significant reduction in the amount of Fe-rich complexes (e.g. PSI) in both strains. However, such a reduction mainly restricts growth in the coastal diatom, but not in the oceanic one, suggesting that in the former, specific bioenergetic adaptations of photosynthesis and respiration allow overpassing Fe limitation. The working hypothesis is that both mitochondria and chloroplasts cooperate to fulfill energy requirements. Electronic microscopy and proteomic analysis are underway to elucidate the mechanisms underlying the different responses to iron limitation in these diatoms and better understand the ecological traits of this essential phytoplankton taxon.

## Five years of high frequency FRRF measurements from the SMILE buoy - Estimation of primary production from hour to year

Claquin, P.<sup>1</sup>, Navon, M.<sup>2</sup>, Serre-Fredj, L<sup>1</sup>, Jacqueline, F.<sup>2</sup>, Izabel, G. & Repecaud, M.

<sup>1</sup>Université de Caen Normandie – CREC- UMR BOREA, France <sup>2</sup>IFREMER, France pascal.claquin@unicaen.fr

Many observation systems of the littoral/coastal ocean are focused on phytoplankton biomass and are poorly focused primary production which correspond to a flux measurements. Remote sensing our chlorophyll a concentration are commonly used to estimate primary production by using stock data of chlorophyll a but results have rarely been validated by in situ measurements. To obtain a precise estimation of primary production, in situ measurements are essential but in coastal ecosystems, primary production deeply varies at very short time scale due to numerous environmental factors which makes its estimation difficult. In the present study, a fully automated FRRF was developed an installed on the smart buoy SMILE in the Bay of Seine (English Channel-France). This system allowed to measure photobiological parameters based on single turn over flash fluorescence measurements every two hour. The measurements were used to estimate the phytoplankton primary production from hourly to annual scale. The system is fully autonomous and is a powerful tool for primary production assessments and photobiology studies in coastal ecosystems. The data acquired over five years are coupled with other high frequency parameters such as nitrates, salinity, light, temperature, which allows us to understand the physico-chemical and biological controls of primary production as well as the effect of extreme events.

### PLENARY

#### Kelp Forests in the Anthropocene

#### Wernberg, T<sup>1,2</sup>

<sup>1</sup>UWA Oceans Institute & School of Biological Sciences, University of Western Australia, Australia <sup>2</sup>Flødevigen Research Station, Institute of Marine Research, Norway thomas.wernberg@uwa.edu.au

Kelp forests are the largest marine biome on Earth, and they form the basis of highly productive ecosystems along more than a quarter of the world's coastlines. Over the past few decades many kelp forests have been in decline as a result of multiple abiotic and biotic drivers, and the IPCC now ranks kelp forests as the temperate marine ecosystem most threatened by climate change. Even though interest in kelp forests has increased in the past decade, our understanding of the benefits people derive from them, as well as our efforts to protect and restore them, lags decades behind other ecosystems. In this talk I will present 1) an overview of the status and trends of the world's kelp forests, including the impacts of warming and marine heatwaves, 2) a recent assessment of the ecosystem services and values provided by kelp forests globally, and 3) new approaches to upscaling restoration and future-proofing kelp forests. Recognition of the value of the services provided by kelp forests will provide an impetus to restore and protect these ecosystems, and help meet international targets concerning ocean accounting and ecosystem assessments.

## SYMPOSIUM 17 "Public-Private partnerships in algal research"

### **KEYNOTE**

#### **3 Phases Towards an Industrial Land-Based Seaweed Cultivation**

Christiansen, E.R.

Pure Algae, Denmark \*info@purealgae.dk

Esben Christiansen, founder of Pure Algae, will be presenting a keynote speech at the conference titled "3 Phases Towards an Industrial Land-Based Seaweed Cultivation." Esben is a biotechnology engineer who has successfully industrialized land-based cultivation systems for seaweed, which capture nutrients and  $CO_2$  while enabling controlled and stable productions.

His passion for bioremediation has led to a profitable and impactful venture that turns emission streams of nitrogen, phosphorus, and  $CO_2$  into a revenue stream. During his presentation, Esben will share his journey navigating a life science startup through different phases of scale, offering tips and tricks for starting and bringing new technology into industrial scale. He will also discuss the challenges and barriers that entrepreneurs in life science startups might face.

### Roadmap towards sustainable growth of the European seaweed industry

Jueterbock, A.<sup>1</sup>, Bruckner, C.<sup>2</sup>, Chapman, A.<sup>3</sup>, Duarte, B.<sup>4</sup>, Engelen, A.<sup>5</sup>, Gauci, C.<sup>1</sup>, Hill, G.<sup>1</sup>, Hoarau-Heemstra, H.<sup>6</sup>, Khatei, A.<sup>1</sup>, Mackintosh, A.<sup>1</sup>, Meland, H.<sup>7</sup>, Nilsen, A.M.L.<sup>1</sup>, Melo, R.<sup>4</sup>, Olsen, L.<sup>8</sup>, Rautenberger, R.<sup>9</sup>, Reiss, H.<sup>1</sup> & Wigger, K.<sup>10</sup>

<sup>1</sup>FBA, Nord University, Norway
<sup>2</sup>Polaralge AS, Norway
<sup>3</sup>Tango Seaweed AS, Norway
<sup>4</sup>MARE—Marine and Environmental Sciences Centre & ARNET – Aquatic Research Network Associated Laboratory, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016 Lisbon, Portugal
<sup>5</sup>CCMAR, University of Algarve, Portugal
<sup>6</sup>FFS?, Nord University, Norway
<sup>7</sup>Norwegian Seaweed Association, Norway
<sup>8</sup>Sjy Seaweed AS, Norway
<sup>9</sup>NIBIO, Norway
<sup>10</sup>HHN, Nord University, Norway

In Europe, the seaweed farming industry has the potential to become a new blue bioeconomy sector. Macroalgae farming is considered inherently sustainable. However, threshold points may be reached under upscaling from experimental cultivation schemes to commercial production. While the number of

liasions are growing that aim to assess sustainability aspects of macroalgal farming, our main goal was to develop what is yet lacking: a roadmap with specific actions that can maximize the potential for a sustainable development of the macroalgal industry in Europe. A secondary goal was to identify angle points that allow for multi-disciplinary research and true collaboration between industry partners and academics.

We first conducted semi-quantitative interviews with representatives from the Norwegian and Portuguese seaweed industry, and then performed a backcasting approach during a workshop with experts from multiple disciplines: ecology, social sciences, and representatives from the industry. Using qualitative data analysis, we identified sustainability aspects that are synergistic across the four sustainability dimensions, economic, ecological, governmental, and societal. We linked these aspects to seven roadmap actions, and to the technology push / market pull concepts.

In our study, we highlight key steps of the roadmap that allow for economical growth of the European macroalgae industry while securing sustainability also in the environmental and social dimensions. Adjusting regulations, multidisciplinary collaboration and research, market development, and assessment of carrying capacities are identified as the most crucial aspects for a fair treatment of future generations that carry the consequences of the actions we take today.

# Studies on the use of locally available (Coxs Bazar and Saint Martin) alternative renewable seaweed wastes as compost organic fertilizer resources

#### Durlave, R.

Masters in sustainable agriculture and rural livelihood Student, 4<sup>th</sup> semester, Bangladesh Open University, Bangladesh kdr2021@gmail.com

Marine red algae from the Bangladesh Bay of Bengal Hypnea Sp have been used as organic materials due to the presence of a number of plant growth-stimulating compounds. The effect of various seaweed species on plant growth and development with an emphasis on the use of this renewable bio-resource in sustainable agriculture of northern fertilizers raw materials system. Organically made fertilizers play an important role in increasing crop yield and the quality of crops promises improvements considering climate adaptation. Seaweed wastes compost was put in evaluation trials at Sreemangal, Bangladesh to evaluate its efficacy and find out the optimum dose for profitable Betel leaf production. This part of the study is directed toward the analysis of the future trend and performances of composting seaweed wastes. The science of seaweeds explores, how analysis of the future trend and performances of composting seaweed wastes. A field study was conducted at three sites at Khasia farmers of Sreemangal Khasia betel leaf cultivation community area of Bangladesh. Seaweed wastes mixed with compost organic fertilizer dose of 50g per support tree. The highest betel leaf yield was obtained from seaweed wastes mixed with compost organic fertilizer applied to plants. Two people have not used it, but the one who has used it has had good results. This study suggests that seaweed wastes mixed with organic fertilizer are suitable for betel leaf cultivation. Area-based conservation is a key tool for delivering the SDG goal of responsible production and consumption.

### KEYNOTE

#### Discovery of the potential of brown seaweed lipid extracts against Alzheimer's disease in a public-private partnership

#### Mulder, M.T.

Laboratory Vascular Medicine, Department Internal Medicine, Erasmus MC University Medical Center, Rotterdam, the Netherlands m.t.mulder@erasmusmc.nl

Algae contain numerous bioactive molecules with health improving properties. This provides opportunities for their application in the prevention and treatment of various diseases.

In collaboration with the private sector we have established a research line on the potential of brown algae containing unique phytosterols in the prevention and treatment of Alzheimer's disease (AD).

Our initial finding was that dietary supplementation of AD mice with the Asian brown seaweed Sargassum fusiforme prevented disease progression via the liver X receptor (LXR)agonist 24(S)-saringosterol. Initially in a joint project with "St Zeeschelp" we identified Dutch seaweeds containing saringosterol. In a public-private partnership with The Seaweed Company we obtained 2 grants to further investigate the potential of local European seaweeds against, and another grant to investigate the potential against type 2 diabetes mellitus in patients. In one of the project Feyecon D&I, specialized in extraction methods, was involved. We investigate the potential of Luropean seaweeds, their lipid extracts and identified pure compounds in the prevention and treatment of AD. Seaweed-derived phytosterols can prevent disease progression in Alzheimer mice via activation of LXR without inducing serious adverse effects as synthetic LXR-agonists do. So far we found six European brown seaweed species: *Alaria esculenta, Ascophyllum nodosum, Fucus vesiculosis, Himanthalia elongata, Saccharina latissimi*, and *Sargassum muticum* to activate LXRs, with the Himanthalia elongata extract showing the strongest effect comparable to *Sargassum fusiforme*. In addition, we identified five LXR-activating oxysterols (2 isolated from *Sargassum fusiforme* and 3 synthesized sidechain-oxidized sterol derivatives) in a collaboration with the Ocean University of China.

The final aim is to develop affordable and sustainable products that can be applied in the prevention and or treatment of AD, in a private-public partnership with The Seaweed Company and with an option to initiate a start-up or spin-outC company.

#### Valorization of marine oxylipins; a story of private-public partnership

Linares-Maurizi, A.<sup>1,2</sup>, Pradelles, R.<sup>2</sup>, Vigor, C.<sup>1</sup>, Bertrand-Michel, J.<sup>3</sup>, Durand, T.<sup>1</sup> & Usache, V.<sup>2</sup>

<sup>1</sup>IBMM - UMR 5247, Pôle Chimie Balard Recherche, 34093 Montpellier, France <sup>2</sup>Microphyt, 713 Route de Mudaison, 34630 Baillargues, France <sup>3</sup>MetaboHUB, Inserm/UPS UMR 1048 - I2MC, Institut des Maladies Métaboliques et Cardiovasculaires, 31077 Toulouse, France amandyne.linares-maurizi@microphyt.eu

To address the challenges of identifying new bioactive compounds from microalgae to supply development of new natural solutions for well-being markets, a private-public partnership, via a CIFRE thesis contract, was created thanks to an ANRT supported collaboration between two experts in their field; Microphyt and IBMM.

Microphyt is a leading " blue " biotechnology company in the research, development, production and marketing of unique natural active ingredients from microalgae for the nutrition and cosmetics sectors. The SLB team at IBMM, CNRS is an expert in the research of bioactive lipids and more particularly on the total synthesis and quantification of oxidized metabolites of polyunsaturated fatty acids in different matrices.

This complementarity allows to lift the scientific and technological barriers of the industrial production in photobioreactor of microalgal biomasses with specific oxylipin profiles. The objective of this collaboration is thus to associate the fine control of the microalgal production and the extraction of the compounds of interest in an optimal way with a characterization and quantification of the oxylipins at each stage.

The first works highlight the interest of microalgae as a source of bioactive lipids that could favor the resolution of inflammation in therapeutics or nutraceuticals. This rich mixture of oxylipins found in these microalgae are promising for human health benefits like antioxidant, anti-inflammatory, antiarrhythmic, neuroprotector and immunomodulator.

This collaboration is the fruit of many very conclusive results, of many scientific communications. Subsequently, this research work can be enhanced and lead to the development of marine products enriched in oxylipins.

#### Algaecenter Denmark – from research to industry

Bruhn, A.<sup>1</sup>, Mouritsen, L.T.<sup>2</sup>, Ma, N.<sup>3</sup> & Thomsen, M.<sup>4</sup>

<sup>1</sup>Aarhus University, Department of Ecoscience <sup>2</sup>The Kattegatcentre, Denmark <sup>3</sup>Danish Technological Institute, Denmark <sup>4</sup>University of Copenhagen, Denmark anbr@ecos.au.dk

The mission of AlgaeCenter Denmark (ACDK) is to generate sustainable innovation in the macroalgae industry – all the way through the value chain from production of seaweeds to end products. The ACDK was founded in 2010 as a science-industrial consortium of cooperation between Aarhus University (AU), The Kattegatcentre (KC) and Danish Technological Institute (DTI). In 2022, University of Copenhagen (UoC) joined as partner.

Complementing competences and open sharing of knowledge and network has enabled ACDK to progress the Danish macroalgae industry: The centre works as an 'open science hub' where the annual international Nordic Seaweed Conference gathers participants from science and industry to discuss and exchange knowledge and knowledge needs in the macroalgae industry sector. Adjacent to the conference, a national industrial network meets to discuss the Danish perspectives of innovation in macroalgae industry and the future strategies. The ACDK facilities for research and development in macroalgae production and processing can be rented - including science support - by industries and SMEs. The macroalgae cultivation facilities, run by AU, include land-based and coastal cultivation systems. The processing facilities, run by the DTI, include pre-processing and biorefinery facilities from lab to pilot scale. The UoC has established a macroalgae production and ecosystem services to pupils and citizens from the age of 5 to 99.

The ACDK associated networks have generated start-up companies, B2B connections, product development, policy making, job opportunities for young scientists and project cooperations driving the future innovation.

## SYMPOSIUM 02 "Biodiversity assessment and algal distribution in space and time"

#### **KEYNOTE**

#### Environmental DNA-based assessment of Charophyte biodiversity: Progress and remaining challenges

Nowak, P.<sup>1,3</sup>, Wiebe, C.<sup>1</sup>, Karez, R.<sup>2</sup> & Schubert, H.<sup>1</sup>

<sup>1</sup>University of Rostock, Germany <sup>2</sup>Landesamt für Umwelt Schleswig-Holstein, Germany <sup>3</sup>Institut für Seenforschung LUBW, Germany petra.nowak@uni-rostock.de

Assessing biodiversity is a central element in biogeography and ecosystem management. However, detecting rare species in large and/or heterogeneous systems is difficult and timeconsuming. Environmental DNA (eDNA) analyses have the potential to provide at least some indication of where rare species might have been overlooked and where more monitoring effort is required and worthwhile. This method has already shown its potential for surveys of animals such as fishes and amphibians, but its applicability to plants and macroalgae still needs to be tested.

Here, we present the results of an approach to applying eDNA analysis for documenting the diversity of charophyte species in different aquatic ecosystems using eDNA markers for a range of chloroplast genome regions. The focus was on charophytes because of their importance as habitat engineers and, consequently, bioindicators for ecological status. In addition, the markers developed here were also tested for their potential to overcome difficulties in morphological determination, which has resulted in underestimations of their species diversity in the past.

The method developed and presented here has successfully identified charophytes down to the species/group level. Regardless of the remaining obstacles discussed, eDNA offers a tool for efficient field surveys that provide targeted and site-specific data to complement or augment conventional monitoring.

# Coralline red algae of Antarctica and Subantarctic regions: state of knowledge of a threatened, yet poorly known, group of ecosystem engineers

Rindi, F.<sup>1</sup>, Trentin, R.<sup>2</sup>, Sciuto, K.<sup>3</sup>, Moschin, E.<sup>2</sup>, Nelson, W.A.<sup>4</sup> & Moro, I.<sup>2</sup>

<sup>1</sup>Dipartimento di Scienze della Vita e dell'Ambiente, Università Politecnica delle Marche, Italy <sup>2</sup>Dipartimento di Biologia, Università di Padova, Italy <sup>3</sup>Dipartimento di Scienze Chimiche, Farmaceutiche ed Agrarie, Università di Ferrara, Italy <sup>4</sup>National Institute of Water and Atmospheric Research, New Zealand

Due to limitations imposed by the extreme environmental conditions, Antarctica and Subantarctic regions remain among the least phycologically known. Corallines of these regions are poorly known, despite a long history of studies dating back to the early twentieth century. Current knowledge of their diversity is based mostly on traditional morphoanatomical investigations. For Antarctica proper, reliable

records are available for 12 species; most of these are based on collections from intertidal and shallow subtidal habitats, with *Leptophytum coulmanicum* and *L. foecundum* being the most frequently reported. However, recent collections made in the deep subtidal of the Ross Sea and studied by a combination of morpho-anatomical and molecular data produced the discovery of the new genera and species *Tethysphytum antarcticum* and *Thalassolithon adeliense*; these represented two previously unknown lineages within the order Hapalidiales. Molecular data suggest that *Thalassolithon* has probably a much wider distribution, extending to the Auckland Islands and New Zealand. While 11 species of corallines are recognized based on molecular data from the Balleny Islands and the New Zealand subantarctic islands, only 5 of these can currently be assigned to genera. Four of them are confined to the Ross Sea/Balleny Islands or subantarctic islands, while the remainder have much wider distributions to Rakiura/Stewart Island and the New Zealand mainland. These discoveries confirm that the knowledge of coralline diversity in the Southern Ocean is largely incomplete. Further collections from presently unexplored regions and use of DNA sequence data, including sequences obtained from relevant type specimens, are key requirements for future progress.

#### A Red List for British Seaweeds

Wilbraham, J.<sup>1</sup>, Yesson, C.<sup>2</sup> & Brodie, J.<sup>1</sup>

<sup>1</sup>Natural History Museum, London, UK <sup>2</sup>Zoological Society of London, London, UK

Seaweeds are a critical part of the British coastline, forming vital habitats and providing valuable ecosystem services. They are also under threat from warming seas, potential impact from harvesting, loss of habitats and increases in the arrival of non-native species. There has never been a greater need to conserve seaweed diversity and to determine those species which are at risk of decline. The IUCN Red List of Threatened Species is an authoritative tool in biodiversity conservation which is used to assess the risk of extinction and here we report on recent work to produce the first Red List for British Seaweeds. Whilst the IUCN criteria are intended to be objectively applicable to all species of plants and animals, particular challenges were found in applying these to seaweeds. Reasons include inconsistent recording, few recorders, difficulty identifying species, under sampled hard to access habitats and complex life histories. Observation records from national and regional sources were used to apply IUCN criteria using a data driven approach which categorized 13% of 617 British seaweeds as threatened. A review panel to add expert weighting to these results reduced this to 7% of species placed in threatened categories, with a further 9% listed as near threatened. A notable c. 55% of the 617 species evaluated were classed as data deficient. Recommendations from this study include a more strategic approach to future data collection, and consideration of extending the Red List assessment to the wider North East Atlantic region, involving a wider network of seaweed specialists.

### SYMPOSIUM 07 "Genomics technologies in algae"

#### **KEYNOTE**

#### **Genomics and Reverse Genetics in Diatoms**

Mock, T., Belshaw, N. & Hopes, A.

School of Environmental Sciences, University of East Anglia, Norwich Research Park, United Kingdom t.mock@uea.ac.uk

Diatom research has experienced a step change over the past decade due to the development of genomics and reverse genetics tools. This presentation will not only provide insights into the latest developments, but it will also address challenges with respect to the complexity of diatom genomes especially in the context of synthetic biology and genetic engineering technologies. Thus, we will discuss the latest sequencing technologies and how to use CRISPR/Cas-based genome editing for efficient genome engineering in diatoms. This fundamental knowledge is required because of growing interest to work with diatom species that have attributes not covered by the most used models such as *Thalassiosira pseudonana* and *Phaedactylum tricornutum*.

### ORALS

### The genome of *Palmaria palmata*: a tool to advance seaweed strain selection and unveil intraspecific variability across Northern Europe

Rosignoli, S.<sup>1</sup>, McHale, M.<sup>1</sup>, Schmid, M.<sup>1</sup>, Edwards, M.D.<sup>2</sup>, Inaba, M.<sup>1</sup>, Fort, A.<sup>3</sup> & Sulpice, R.<sup>1</sup>

<sup>1</sup>University of Galway, Ireland <sup>2</sup>Irish Seaweed Consultancy, Ireland <sup>3</sup>Technological University of the Shannon, Ireland serena.rosignoli@universityofgalway.ie

The rhodophyte *Palmaria palmata* (L.) Weber & Mohr is one of the target species of a growing European seaweed industry due to its high content of protein (up to 35%), essential minerals, bioactive peptides and antioxidants and its uses as food, diet supplement and salmon feed. However, the lack of a nuclear genome limits phylogenetics analyses and gene function investigations which could help the development of a breeding programme.

We present a draft genome of *P. palmata* obtained with PacBio HiFi sequencing with average coverage of 10x, and consisting of an assembly of 1.1 Gb, N50=2Mb and BUSCO completeness of 75%. Additionally, the genome of 32 more *P. palmata* individuals from across the Northern East Atlantic area were sequenced with Illumina and their ongoing variant calling will contribute to reveal the intraspecific adaptation to different environments of this species.

The results will set the bases for strain selection and molecular breeding, to maximize the economic potential and sustainability of *P. palmata* in its various applications.

### Chloroplast DNA methylation in the kelp Saccharina latissima is determined by origin and influenced by cultivation

Scheschonk, L.<sup>1</sup>, Nilsen, A.M.L.<sup>2</sup>, Bischof, K.<sup>1</sup> & Jueterbock, A.O.<sup>2</sup>

<sup>1</sup>University of Bremen, Marine Botany & MARUM, Germany

<sup>2</sup>Nord University, Faculty of Biosciences and Aquaculture, Algal and Microbial Biotechnology division, Norway

anne.m.nilsen@nord.no

We detected methylation in the chloroplast DNA of sugar kelp Saccharina latissima, a nonmodel macroalgal species that is one of the foundations of kelp forests. Furthermore, it is one of the most common species for kelp aquaculture in Europe and North America. In brown algae, little is known about the chloroplast methylome beyond its detection in the congener kelp Saccharina japonica, and its function is yet largely unexplored. Because the chloroplast is highly sensitive to heat stress, its methylome may hold the potential for rapid thermal acclimation. We compared methylation patterns between wild and cultured kelp from different climatic regions: High-Arctic (Spitsbergen, Norway, 79 °N) and temperate (Helgoland, Germany, 53 °N), with cultivated sporophytes grown at 5 °C, 10 °C and 15 °C. Our results suggest genome-wide differences in methylated sites, and methylation levels, between the climatic regions. We also found differential methylation between the wild and cultivated samples. Our findings indicate that both origin and cultivation strongly affected the chloroplast methylome, but differently. Thus, both the origin population and laboratory cultivation may affect epigenetically controlled characteristics. Because some of the same differences between climatic regions remain across both field and lab cultivated samples, our results suggest that population differences have higher importance to the methylome than the recent changes in conditions during the cultivation. The differential methylation of kelps from the High Arctic between field and lab samples could have implications for the Arctic kelp's capacity for rapid acclimation to environmental stressors.

### **KEYNOTE**

### Phytomyxea use conserved host physiological properties to induce a sink and to avoid host defence

Neuhauser, S.<sup>1</sup>, Garvetto, A.<sup>1</sup>, Hittorf, M.<sup>1</sup> Kirchmair, M.<sup>1</sup>, Murua, P.<sup>2</sup> & Gachon, C.M.M<sup>3</sup>

<sup>1</sup>Institute of Microbiology, University of Innsbruck, Technikerstr. 25, 6020 Innsbruck, Austria <sup>2</sup>Laboratorio de Macroalgas, Instituto de Acuicultura, Universidad Austral de Chile, Puerto Montt, Chile <sup>3</sup>Muséum National d'Histoire Naturelle, UMR 7245, CNRS CP 26, 57 rue Cuvier, 75005 Paris, France sigrid.neuhauser@uibk.ac.at

Phytomyxids (Rhizaria, Endomyxa, Phytomyxea) are parasitic biotrophs that depend on host plants, brown algae, diatoms, and oomycetes. Parasites belonging to this group are abundant in both terrestrial (soil and fresh water) and marine habitats, and their diversity, specifically in the marine environment is yet to be fully explored. Phytomyxids are inseparable from their hosts; hence phytomyxid biology and their interactions with the host require a well selected combination of different novel and traditional approaches. Despite the advanced genetic modifications of plant hosts, such techniques are not yet available for phytomyxids or are still nascent in brown algal hosts. To address this, we began to examine the transcriptomes of phytomyxid-infected plants and algae as well as the patterns of expression of

certain genes involved in the interaction between the parasite and the host using FISH techniques, TEM microscopy, and other techniques. With the use of these combinations of techniques, we were able to confirm that these parasites receive at least some of their energy from their hosts via a specialized form phagotrophy which is seen in genomic and morphological observations. Moreover, we were able to establish how crucial endoreduplication is in the pathogen's ability to cause cell cycle arrest. The pathogen induced the strengthening of the cell walls of the algal hosts in response to the early stages of infection, which we could demonstrate. Overall the combination of these fundamentally different approaches lead to an increase in our understanding of the host-pathogen interaction.

### ORALS

### Carbon-neutral, eco-friendly polymer from microalgae and cyanobacteria

### Ramanan, R.<sup>1,2</sup>, Kim, J.W.<sup>1,3</sup>, Kim, H-S.<sup>1,3</sup>

<sup>1</sup>Cell Factory Research Centre, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Republic of Korea

<sup>2</sup>Sustainable Resources Laboratory, Department of Environmental Science, Central University of Kerala, India <sup>3</sup>Department of Environmental Biotechnology, KRIBB School of Biotechnology, Korea University of Science and Technology (UST), Republic of Korea rishi@cukerala.ac.in

Plastic debris is an established environmental menace affecting ecosystems globally. With the current rate of manufacture and disposal, plastic litter will increase 10-fold over the next ten years, not accounting for COVID-19-induced single-use plastics disposal recently, and further exacerbate adverse impacts on environmental and human health. Nearly all plastics produced globally are fossil fuel derived. Therefore, there is a need for a carbon-neutral, biodegradable polymer, preferably from biomass which could address all spheres of sustainability. Poly-3-hydroxybutyrate (PHB) is a polyester produced by bacteria and cyanobacteria, which has the potential to replace present-day plastics. However, largescale cultivation of PHB-producing organisms remains a techno-economic challenge because of a host of issues such as carbon source, shear stress during cultivation, and overall cost of production. Moreover, microalgae have not been proven to produce PHB, and none of the genes associated with PHB production or degradation in microalgae are characterized. In this study, we used a novel CRISPR-Cas9 approach to characterize genes associated with PHB molecules in microalgae. The associated enzymes in microalgae are characterized along with the CRISPR-Cas9generated mutants. In parallel, novel, PHBproducing cyanobacterial strains suited for mass cultivation have been prospected. Hence, the study characterizes novel enzymes, microalgal mutants, and cyanobacterial strains involved in PHB synthesis, ultimately leading to carbon-neutral, eco-friendly polymer from carbon dioxide.

### Diatom phytochromes: solving the riddle of a red-light sensor in the red-poor marine environment

Duchêne, C.<sup>1,4</sup>, Bouly, J-P.<sup>1</sup>, Pierella Karlusich, J.J.<sup>2</sup>, Sellés, J.<sup>1</sup>, Bailleul, B.<sup>1</sup>, Bowler, C.<sup>2</sup>, Ribera d'Alcalá, M.<sup>3</sup>, Falciatore, A.<sup>1</sup> & Jaubert, M.<sup>1</sup>

<sup>1</sup>Chloroplast biology and light-sensing in microalgae, Institut de Biologie Physico-Chimique, Paris, France <sup>2</sup>Plant and algal genomics, Département de Biologie, École Normale Supérieure, Paris, France <sup>3</sup>Stazione Zoologica Anton Dohrn, Naples, Italy <sup>4</sup>Current address: Department of Algal Development and Evolution, Max Planck Institute for Biology Tübingen, Tübingen, Germany carole.duchene@tuebingen.mpg.de

Light gradients along the watercolumn shape life in the oceans. Indeed, the light field changes in both intensity and quality with depth, with strong attenuation of long wavelengths such as red (R) and farred (FR). Surprisingly, predominant marine algae such as diatoms have phytochromes photoreceptors, which are red/far-red receptors in land plants. Diatom phytochromes (DPH) exhibit R/FR absorption spectra and induce expression of a set of genes upon FR light exposure. How a R/FR receptor is activated in the red-poor marine environment remains a puzzle. Here we propose an answer with a new model for phytochrome activation in the ocean. By measuring the responses triggered by DPH photoreceptor *in vivo* thanks to a reporter system, we showed that its action is not restricted to the long R/FR wavebands, but extends to the entire visible light spectra. Considering these new properties, FR light is ineffective in triggering phytochrome responses in an oceanic context, where phytochrome instead detects the balance between other wavebands (particularly red, green and blue), that reflect depth variations and the presence of other photosynthetic organisms. This paradigm shift in phytochrome sensing abilities opens up new insights into the role of phytochromemediated light sensing in the oceans.

## SPECIAL SESSION "Publishing phycological papers in a rapidly changing environment"

#### Brodie, J.<sup>1</sup> & Maggs, C.A.<sup>2</sup>

<sup>1</sup>Natural History Museum, Cromwell Rd, London SW7 5BD <sup>2</sup>School of Biological Sciences, Queen's University, Belfast BT9 5AJ, N. Ireland j.brodie@nhm.ac.uk

Navigating the current world of publishing at the start of a scientific career can be challenging. The current major transition from traditional publishing to the increasing importance of Open Access (OA) journals raises a number of issues, particularly for learned society based journals, when the funding model for OA is not yet fully agreed. For some journals, and in some countries, publishers are increasingly developing Transitional Agreements (TAs) with both nations and institutions at a range of levels (e.g. libraries). Consequently there is a move away from traditional institutional subscriptions to journals, many of which are partnerships between publishers and learned societies. Transitional Agreements will allow authors in the institutions to publish without manuscript-handling charges. In this session, we will address OA, as well as predatory journals, paper mills, and special issues. It will also provide an opportunity for a wider discussion on getting published and the role of learned societies. We will encourage greater involvement of early career scientists in journals by reviewing and joining editorial boards.

### SYMPOSIUM 12 "Polar algae in a changing environment"

#### KEYNOTE

### Biosphere – Cryosphere interactions during periods of rapid environmental change

Williamson, C.J.<sup>1</sup>, Bowles, A.<sup>1</sup>, Millar, J.L.<sup>1</sup>, Broadwell, E.L.<sup>1</sup>, Lewis, M., Donoghue, P.J.C.<sup>1</sup>, Williams, T.A.<sup>1</sup> & Campbell, D.<sup>2</sup>

<sup>1</sup>MicroLab@Bristol Research Group, Bristol Glaciology Centre, School of Geographical Sciences, University of Bristol, UK. <sup>2</sup>Department of Biology, Mount Allison University, New Brunswick, Canada.

c.williamson@bristol.ac.uk

Glacial systems are in rapid decline worldwide, with significant perturbations expected in the microbial 'living skin' of glacier surfaces over the coming peak melt century. Primarily dominated by Streptophyte "glacier algae", these microbial communities perform critical roles in carbon/nutrient cycling and form important ice-albedo feedbacks that exacerbate glacier wastage. They also represent the last remnants of ancient ice-dwelling communities that evolved millions of years ago and serve as important analogs for life on other icy worlds across our solar system.

Here I report the latest findings from the "iDAPT" project that studies glacier algal blooms across the cryosphere. By combining in-situ fluorescence imaging, microprobe sensing, and field microscopy we have derived new insight into glacier algal ecophysiology. Coupled with modelling efforts, these data have revealed climate-driven expansion of glacier algal blooms over the past two decades as new regions of the cryosphere have become available for colonization. Genome sequencing, phylogenetic and molecular clock analyses have confirmed the placement of glacier algae within the sister lineage to land plants and highlighted their evolution during the Cryogenian; intonating a role for the glacial biosphere during mass glaciation events. Glacier algal genomes have also provided new insight into the processes of land plant terrestrialization, revealing important algal exaptations for the colonisation of land and patterns of gene novelty and expansion in the ancestor of land plants.

Such studies remain critical as human actions continue to dismantle glacial systems, eliminating species, genes and biological traits that may not be found anywhere else on Earth.

### ORALS

### Under-ice bloom: temporal dynamics and biogeography of Arctic sympagic algae and phytoplankton\*

Sim, C.W.H.<sup>1</sup>, Gérikas Ribeiro, C.<sup>2</sup>, Marie, D.<sup>3</sup>, Probert, I.<sup>3</sup>, Gourvil, P.<sup>3</sup>, Vaulot, D.<sup>3</sup> & Lopes dos Santos, A.<sup>1</sup>

<sup>1</sup>Asian School of the Environment, Nanyang Technological University, 50 Nanyang Avenue, 639798, Singapore.

<sup>2</sup>GEMA Center for Genomics, Ecology & Environment, Universidad Mayor, 8580745, Santiago, Chile.

#### FRIDAY 25<sup>th</sup> August 2023 Room « Tali friz »

<sup>3</sup>CNRS, Sorbonne Université, CNRS, UMR 7144, ECOMAP, Station Biologique de Roscoff, 29680, Roscoff, France.

simw0034@e.ntu.edu.sg

The Arctic is one of the most productive regions in the world, owing to the annual phytoplankton spring bloom which develops when light becomes seasonally available. The open water phytoplankton bloom is preceded by an under-ice bloom. To understand the temporal dynamics of these communities, a time series was conducted from April to July 2016 on land-fast ice off the west coast of Baffin Bay. Size-fractionated sea-ice algae and eukaryotic phytoplankton communities were analysed using metabarcoding of the V4 region of the 18S rRNA gene. Sympagic and planktonic photosynthetic communities were dominated by diatoms (Bacillariophyta). Micro- (20-100  $\mu$ m) and nanophytoplankton (3-20  $\mu$ m) communities underwent a pennate-centric-pennate diatom succession through the different stages of bloom. Pico-phytoplankton (0.2-3  $\mu$ m) was initially dominated by Bolidophyceae followed later by the chlorophyte *Micromonas polaris* and the haptophyte *Phaeocystis pouchetii*.

Biogeographical distribution of key ribotypes (ASVs) retrieved from ice and water were determined from a global analysis of more than 2,500 metabarcoding samples available from the metaPR2 database. Ice algae ribotypes were distributed across ice-covered seas (polar or polar-temperate), reflecting their dependence on sea ice habitat. Some key phytoplankton ribotypes were not restricted to the Arctic, but exhibited polar-temperate or cosmopolitan distributions, suggesting that Arctic spring bloom communities harbour ribotypes from nonArctic waters, which may take over in the future and alter the phenology of blooms with consequences for the whole ecosystem.

### Single-cell elemental profiling of polar snow algae to investigate cellular responses to low nutrient availability in the cryosphere

Feord, H.K.<sup>1</sup>, de Oliveira, S.R.<sup>2</sup>, Lopes Júnior, C.A.<sup>3</sup>, Peters, R.S.<sup>1</sup>, Benning, L.G.<sup>1,4</sup> & Meermann, B.<sup>2</sup>

<sup>1</sup>German Research Centre for Geosciences – GFZ, Potsdam, Germany <sup>2</sup>Federal Institute for Materials Research and Testing - BAM, Berlin, Germany <sup>3</sup>Department of Chemistry, Federal University of Piauí - UFPI, Teresina, Piauí, Brazil <sup>4</sup>Department of Earth Sciences, Free University of Berlin, Berlin, Germany helen.feord@gfz-potsdam.de

Cryophilic chlorophyte algae bloom on oligotrophic snow fields globally, yet little is known about how different species of snow algae thrive in nutrient-poor conditions. Using laboratory cultures of snow algae, we characterised the total single-cell concentrations of target elements (P, Mg, Ca, Zn, Cu, Mn, and Fe) under nutrient-replete conditions, and show how these elemental profiles change under nutrient starvation. We used a novel multielemental singlecell ICP-ToF-MS method, and quantified changes in ion mass per cell for three cultures of polar snow algae (*Microglena sp., Raphidonema sp.*, and *Deuterostichococcus sp.*) and compared these to the mesophilic chlorophyte model *Chlamydomonas reinhardtii*. FlowCam measurements were also undertaken to match elemental concentration distributions to biovolume distributions within a given sample. Overall, our results document a significant elemental cell-to-cell variation in all tested species and for all elements, highlighting the relevance of such single-cell measurements in contrast with traditional bulk ICP-MS analysis. In nutrient-replete conditions, the elemental profiles of each of the four species were distinct and separated well on a PCA plot, with the snow algae exhibiting lower Ca content compared to *Chlamydomonas*. We show that 5 days of P starvation for *Microglena sp.* caused a halving of intracellular P, while for

*Raphidonema sp.* this treatment did not lead to any significant effect on P. These different responses point to diverse adaptative mechanisms to nutrient limitation among snow algae species. Ongoing work includes testing this single-cell method with environmental samples from Greenland and Iceland to match single-cell ICP-ToF-MS data to environmental ion availability.

### Heading northwards? *Laminaria hyperborea* in the Arctic – past, present and future

Diehl, N.<sup>1,2</sup>, Bartsch, I.<sup>2</sup>, Bligh, M.<sup>3</sup>, Buck-Wiese, H.<sup>3</sup>, Hehemann, J.-H.<sup>1,3</sup>, Laeseke, P.<sup>1</sup>, Niedzwiedz, S.<sup>1</sup>, Plag, N.<sup>4</sup>, Shan, T.<sup>5</sup>, Trautmann, M.<sup>1</sup> & Bischof, K.<sup>1</sup>

<sup>1</sup>University of Bremen & MARUM, 28359 Bremen, Germany
 <sup>2</sup>Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research, 27515 Bremerhaven, Germany
 <sup>3</sup>Max Planck Institute for Marine Microbiology, 28359 Bremen, Germany
 <sup>4</sup>Institute of Biological Sciences, University of Rostock, 18059 Rostock, Germany
 <sup>5</sup>Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071, China
 ndiehl@uni-bremen.de

Due to global rise of temperatures, recent studies predict an expansion of several species to higher latitudes. One of these is the boreal-temperate kelp Laminaria hyperborea (Phaeophyceae). It is a dominant and important European bioengineering species that is widely distributed between northern Portugal and northern Norway. The species thrives best at 10-15°C, but can generally survive temperatures between 0 and 21°C. As 'season anticipator', L. hyperborea grows at low temperatures and during the short days of winter and early spring. It has been shown in different single-factorial experimental set-ups to survive several months of cold temperatures and darkness. Therefore, it is striking that L. hyperborea has not yet spread throughout the high Arctic. To investigate potential ability to invade high Arctic sites under future warming scenarios, we conducted a long-term multifactorial experiment using tissue of adult sporophytes of L. hyperborea collected from Porsangerfjorden, Norway - a site close to the species' northernmost distribution margin. We monitored their physiological conditions, namely photosynthetic activity and dry weight, over three months under polar night, polar day and long-day conditions at 0, 5 and 10°C. In addition, we quantified their biochemical composition, such as carbohydrates, pigments and antioxidants. In this way, we aim to reveal strategies for L. hyperborea to acclimate to past, present and future conditions in the Arctic. Will the species spread northwards as climate change continues?

### Seasonal changes in photosynthesis and biochemical composition in Arctic macroalgae undergoing a climatic transition

Carmona, R.<sup>1</sup>, Iñiguez, C.<sup>1</sup>, Smerdou, C.<sup>1</sup>, Gordo, E.<sup>2</sup>, Cañete, S.<sup>2</sup>, Macías, M.<sup>1</sup> and Gordillo, F.J.L.

<sup>1</sup>Department of Ecology, Faculty of Sciences, University of Málaga, Spain <sup>2</sup>SCAI, Central Research Facilities, University of Málaga, Spain rcarmona@uma.es

Seasonal physiology of algal community in Kongsfjorden ecosystem is expected to be affected by Global Climate Change. We characterized the photosynthetic performance and biochemical composition

of five common macroalgal species of Kongsfjorden, from early autumn 2016 to late summer 2017. The studied species were the ochrophytes *Saccharina latissima* and *Alaria esculenta*, the rhodophytes *Phycodrys rubens* and *Ptilota gunneri*, and the chlorophyte *Monostroma* aff. *arcticum*.

Fluorescence results endorsed higher values of maximum quantum yield ( $F_{v}/F_{m}$ ) and electron transport rates (ETR<sub>max</sub>) in brown and green species. Decrease in ETR<sub>max</sub> and saturation irradiance in brown and green algae in summer suggest more sensitivity to continuous radiation than in rhodophytes. Photosynthetic parameters from O<sub>2</sub> measurements showed a better photosynthetic performance of ochrophytes in March, under increasing light conditions, while red and green species did in September. In general, <sup>14</sup>C fixation at saturating light was higher in September, except for *A. esculenta* that was in March. The loss of photosynthetic capacity of macroalgae in summer could be attributed to a decrease in pigment concentration, except for *M. arcticum*. In August, brown and green species lipids presented minimum values in March and proteins did not show a clear pattern. In general, higher N and C contents in March and August, respectively, reveal a seasonal pattern in elemental composition, related to nutrient and light availability along the year. Seasonal responses are species-specific and likely related to their particular adaptive features to Arctic environment.

### Inorganic carbon uptake strategies in Antarctic seaweeds: implications for seaweed resilience to ocean acidification

Fernández, P.A.<sup>1</sup>, Palma, G.<sup>2</sup>, Schmider, A.<sup>2</sup>, Macaya, E.<sup>3,4</sup>, Hurd, C.<sup>5</sup>, Amsler, C.<sup>6</sup> & Buschmann, A.H.<sup>1</sup>

<sup>1</sup>Centro i~mar, CeBiB, MASH, Universidad de Los Lagos, Camino a Chinquihue km6, Puerto Montt 580000, Chile.

<sup>2</sup>Centro i~mar, Universidad de Los Lagos, Camino a Chinquihue km6, Puerto Montt 580000, Chile.

<sup>3</sup>Universidad de Concepción, departamento de Oceanografía, Concepción, Chile.

<sup>4</sup>Centro FONDAP de Investigación en Dinámica de Ecosistemas Marinos de Altas Latitudes (IDEAL), Valdivia 5090000, Chile

<sup>5</sup>Institute for marine and Antarctic Studies (IMAS), University of Tasmania, TAS 7001, Australia.

<sup>6</sup>University of Alabama at Birbingham, department of biology, Alabama 35233, US.

pamela.fernandez@ulagos.cl

OA is predicted to have wide-ranging effects on Antarctic marine ecosystems, influencing both calcifying and non-calcifying marine organisms, including seaweeds. Almost all seaweeds can take up CO<sub>2</sub> via passive diffusion, but 70% can additionally utilize HCO<sub>3</sub><sup>-</sup> as a inorganic carbon (Ci) source, and have developed specific mechanisms to increase [CO<sub>2</sub>] around Rubisco, termed carbon concentrating mechanisms (CCMs). In this study, we identify and characterize the CCMs in Antarctic seaweeds across taxonomic groups, and an emersion gradient from the high intertidal to the deep subtidal. A total of 45 species were collected during the austral summer at Fildes Bay, King George Island (62°14'S, 58°40′W). Immediately after sampling, thallus pieces of 0.5 g FW were cut from the meristematic zone. Samples used for pH drift were pre-incubated in natural seawater (SW) for 24 h at 2°C. Simultaneously, samples for stable isotopes and carbon and nitrogen content were oven dried for 48 h at 60°C, then pulverized and stored until further analysis. pH Drift experiments (n=5 per specie) were conducted at 2°C under a saturating light intensity for 12 h. SW pH was recorded at the start and end of the incubation. Results from pH drift experiments and  $\delta^{13}$ C values were highly correlated showing that CCMs varied across taxonomic groups and the emersion gradient. Most of the brown and green species have developed CCMs, while red species, especially those from deeper waters, rely more on CO<sub>2</sub>. Patterns in species CCMs will be crucial to understand how Antarctic seaweed communities will respond OA.

### Influence of recent warming on kelp forest structure in Kongsfjorden (western Svalbard) over time

Düsedau, L.<sup>1,2</sup>, Fredriksen, S.<sup>3</sup>, Brand, M.<sup>4</sup>, Fischer, P.<sup>4</sup>, Karsten, U.<sup>5</sup>, Bischof, K.<sup>2</sup> & Bartsch, I.<sup>1</sup>

<sup>1</sup>Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research, Bremerhaven, Germany
 <sup>2</sup>Marine Botany, University of Bremen, Bremen, Germany
 <sup>3</sup>Department of Biosciences, University of Oslo, Oslo, Norway
 <sup>4</sup>Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research, Helgoland, Germany 5 University of Rostock, Rostock, Germany
 luisa.duesedau@awi.de

The Arctic Svalbard archipelago is one hotspot of global warming and Kongsfjorden (W- Svalbard) experiences a continuous increase in seawater temperature, glacial melt, freshwater run-off and sedimentation. In 1996/98, 2012-14 and 2021 seaweed biomass, species diversity and depth distribution of kelps were quantified at our study site in order to identify potential changes over time. We repeated the earlier studies by stratified random sampling along a sublittoral depth transect (0, 2.5, 5, 10 and 15 m;  $1x1 \text{ m}^2$  frames; n=3) and documented the lower depth distribution of kelps at 2-20 m depth. With 21 biomass dominant species collected in 2021, the biodiversity along the depth transect remained stable. In contrast, the lower distribution limit and the relative abundance of all kelp species severely decreased. Between the 1<sup>st</sup> and 2<sup>nd</sup> study, the biomass maximum doubled and shifted upwards from 5m to 2.5m depth. Although this pattern did not change in 2021, the community structure shifted from a Laminaria digitata/Hedophyllum nigripes based community to an Alaria esculenta dominated kelp forest. In 2021, Alaria esculenta was the only kelp species present at 10m depth while the other kelps retreated to shallower waters. Comparison of kelp demography in 2013 and 2021 revealed a change in dominance relationships between age classes. Presently, a kelp forest with a balanced age distribution is only found at 2.5m depth. Our time series reveals consequences of increasing turbidity and decreasing light availability for Arctic kelp forests in an environment in transition.

### KEYNOTE

### Distant, Yet the Same : How do Arctic and Antarctic Green Algae Cope with Multiple Stressors?

#### Cvetkovska, M.

Department of Biology, University of Ottawa, Canada \*mcvetkov@uottawa.ca

Despite year-round freezing temperatures, the Arctic and Antarctic house a large biodiversity of algae that contribute nearly half of global  $CO_2$  sequestering through photosynthesis. Many polar algae are obligate cold extremophiles (psychrophiles) that thrive at low temperatures but are sensitive to mild heating and perish at temperatures >15°C. More than a third of all confirmed algal psychrophiles belong to the order Chlamydomonadales (Chlorophyta), a group characterized by many established models for algal physiology and molecular biology. We take advantage of this excellent opportunity for comparative analyses of closely related *Chlamydomonas* species from diverse Arctic and Antarctic environments.

The Polar Oceans warm about twice the rate of temperate regions, but the environmental effects are often complex. Even small changes in air temperature can greatly decrease the thickness and duration of the ice cover in polar aquatic ecosystems. Ice thinning leads to decreased salinity due to freshwater influx and increased light availability within the water column. Our work focuses on two questions: 1) How do psychrophilic green algae respond to multiple environmental stressors? and 2) Does initial habitat stability affect robustness in the face of change? We examine the role of salinity and light intensity on the growth and stress physiology of algae from polar habitats, ranging from very stable (e.g., perennially icecovered lakes) to very dynamic environments that experience drastic annual changes (e.g., marine waters, snow fields). Our work provides some of the first insights into how polar algae from diverse habitats respond to a changing climate at a physiological level.

### Arctic *Phaeocystis pouchetii* benefits stronger from warming than from changes in light intensity or pCO<sub>2</sub>

Rokitta, S.D.<sup>1</sup>, Grossmann, C.H.<sup>1</sup>, Werner, E.<sup>1</sup>, Moye, J.<sup>1</sup>, Castellani, G.<sup>2</sup>, Nöthig, E-M.<sup>2</sup> & Rost, B.C.<sup>1,3</sup>

<sup>1</sup>Alfred-Wegener-Institute – Helmholtz-Zentrum for Polar- and Marine Research, Marine Biogeosciences, Am Handelshafen 12, 27570 Bremerhaven, Germany

<sup>2</sup>Alfred-Wegener-Institute – Helmholtz-Zentrum for Polar- and Marine Research, Polar Biological Oceanography, Am Handelshafen 12, 27570 Bremerhaven, Germany

<sup>3</sup>Universität Bremen, FB2, Leobener Strasse, 28359 Bremen, Germany Sebastian.Rokitta@awi.de

In the Arctic, Global warming is much more pronounced than in other regions, and the associated seaice decline and Ocean Acidification lead to higher insolation and changed chemistry. The combination of these co-varying environmental drivers may profoundly impact the ecophysiology of primary producers and the regional biogeochemistry. The haptophyte microalga *Phaeocystis pouchetii* is a key species in the Arctic, where it forms large blooms and often enhances vertical carbon export by promoting organic matter aggregation.

Here, we cultured single cells in a matrix of temperature (2°C vs. 6°C), light intensity (55 vs. 160  $\mu$ mol photons m<sup>-2</sup> s<sup>-1</sup>) and pCO<sub>2</sub> (400 vs. 1000  $\mu$ atm CO<sub>2</sub>). Next to acclimation parameters (growth rates, particulate and dissolved organic C and N, chlorophyll *a*), we measured physiological processes *in-vivo* (electron transport rates and net photosynthesis) using fast-repetition rate fluorometry and membrane-inlet mass spectrometry.

Within the applied driver ranges, elevated temperature had the most pronounced impacts, significantly stimulating growth, elemental quotas and photosynthesis. Light stimulations manifested prominently under 6°C, underlining temperature's role as a 'master-variable'. In comparison, pCO<sub>2</sub> exerted mostly insignificant effects. The obtained data were used for a simplistic upscaling simulation to investigate potential changes in *P. pouchetii*'s bloom dynamics in the Fram Strait with increasing temperatures over the 21<sup>st</sup> century. Results from experiments and simulations suggest that global warming could accelerate bloom dynamics, with earlier onsets of blooms and higher peak biomasses. Hence, increasing temperatures will likely accelerate the phenology of *Phaeocystis* and probably other Arctic phytoplankton with potential implications for higher trophic levels.

### Challenged growth and performance across changing arctic seasonal photoperiods in the polar diatom *Fragilariopsis cylindrus*

Guérin, S.<sup>1</sup>, Bruyant, F.<sup>1</sup>, Gosselin, M.<sup>2</sup>, Babin, M.<sup>1</sup> & Lavaud, J.<sup>1,3</sup>

<sup>1</sup>Takuvik, Canada <sup>2</sup>ISMER-Institut des sciences de la mer de Rimouski, Canada <sup>3</sup>LEMAR-Laboratory of Environmental Marine Sciences, France sebastien.guerin@takuvik.ulaval.ca

Arctic microalgae are exposed to dramatic seasonal changes in light availability, from continuous summer days to winter nights, coupled with often seasonal snow/ice cover changes. Under this harsh light climate, large spring blooms of diatoms occur at the sea-ice bottom and under the sea-ice, accounting for a significant proportion of the annual marine primary production in the Arctic Ocean. The ongoing environmental change in the Arctic drive diatom blooms earlier in spring and later in fall, thus under a wider range of day lengths. Elucidating the response of Arctic diatoms to photoperiods and the mechanisms regulating their daily photosynthetic rhythms will provide a better understanding of the possible consequences of the changing Arctic light climate on the marine primary production. Here, we characterized the response of Fragilariopsis cylindrus, a model polar diatom, through several seasonal Arctic photoperiods, and characterised the observed photoperiodic rhythm in photosynthetic performance. Our results highlight the ability of F. cylindrus to grow efficiently under a wide range of day lengths. We also report circadian regulation of many aspects of photochemistry and photoprotection relying on light-dark alternation. Fine-tuning the balance between photochemistry and photoprotection along with circadian synchronization allows F. cylindrus to make the most of available light to support sustained production and growth from early spring through late winter fall in the Arctic despite the variable day length, low light, and low temperature (ca. 0°C).

### SYMPOSIUM 18 "Algae and the general public" + SYMPOSIUM 19 "Algae, education and career pathways"

### **KEYNOTE**

### Mentoring Undergraduate and Graduate Students for Careers Inside and Outside Academia

#### Vis, M.L.

Department of Environmental and Plant Biology, Molecular and Cellular Biology Program, College of Arts and Sciences, Ohio University, Athens OH USA vis-chia@ohio.edu

An important component of our profession as university professors is mentoring both undergraduate and graduate students. We interact with most undergraduate students in the classroom teaching traditional courses. COVID-19 required us to pivot to new technologies and rethink our classrooms for online learning. Now that we are back to in-person teaching, are there components of our online courses that might be coopted to support our learning outcomes? As an example, I developed, along with my graduate student, an exercise assessing harmful algal blooms from web sources. This project promotes working collaboratively, data mining, statistical analyses, and synthesis of information. These skills are highly valued by employers across industries as well as graduate programs. Not only do we interact with undergraduates in the classroom, but also with a subset in our laboratories as undergraduate researchers. I strive to provide those students with a variety of skills to augment their resumes including bench techniques, data management, problem solving, and written/oral communication. These skills ready the students for graduate school, but also are transferable to industry and government careers. In addition to undergraduates, we mentor graduate students which is a substantial time commitment and responsibility. Most graduate students enter their program having developed the aforementioned skills but need to hone them, especially problem-solving, synthesis and communication skills. New skills need to be added such as independent thinking and mentorship. In my presentation, I will provide my strategies for mentoring students to achieve the skills they need to be successful inside and outside academia.

### Developing citizen science monitoring capacity for adaptation to brown seaweed influxes

Dominguez Almela, V., van der Plank, S. & Tompkins, E.

School of Geography and Environmental Science, University of Southampton, Southampton, UK v.dominguez-almela@soton.ac.uk

Climate change exacerbates known hazards and leads to novel challenges which can have significant societal impacts. Among these challenges is the invasive *sargassum* seaweed, which has emerged as a concern across the tropical Atlantic, including the Western Region of Africa. Limited knowledge exists on how citizen science can foster adaptive capacity in communities affected by brown seaweed influxes. Our study examines community-led monitoring of sargassum in Ghana as a potential means to enhance societal adaptive capacity.

We aim to determine how citizen science can bolster household and community-level adaptive capacity to brown seaweed influxes. In western Ghana, we installed three coastal monitoring stations featuring stainless steel phone holders and collaborated with local communities and schools to identify suitable locations and usage. We provided training for local school children on using data to monitor sargassum and offered information about the seaweed in the form of lessons, which are being used within the science curriculum of the schools.

By interviewing community members, we explored how the monitoring stations influenced their relationship with sargassum, its impact on daily life, and potential adaptive strategies to prepare, respond, and learn about the seaweed. Our findings suggest that while citizen science may not fundamentally alter individual and community capacity to adapt to brown seaweed influxes, it serves as a crucial initial step. This approach augments local and academic knowledge of new threats, such as sargassum influxes, and equips future generations with the tools to understand and address these challenges.

#### Phycophagy and the rise of the phycogastronomy in Europe

Pérez-Lloréns, J.L., Hernández, I., Vergara, J.J. & Brun, F.G.

Instituto Universitario de Investigación Marina (INMAR), Universidad de Cádiz, Av. República Saharaui s/n. 11510-Puerto Real (Cádiz, Spain) joselucas.perez@uca.es

Seaweeds have a rich history as a staple food for people all over the world, especially in Asian countries, notably Japan, China and Korea, where seaweeds have been considered a delicacy since ancient times. Even today they are an important part of their daily menu. In contrast, in southern Europe seaweeds have not been consumed historically, and in northern Europe their consumption still has, to some extent, connotations with feeding the poor and at times of famine and hardship, surviving even in a few scattered places (e.g., Brittany, Ireland or Iceland). Since the mid-2000s, this situation changed in most European countries, when seaweed joined the kitchens of some of the most famous Michelin-starred restaurants run by celebrity chefs (e.g., René Redzepi at Noma, Denmark or Ferran Adrià at El Bulli, Spain) interested in novel uses of seafood, as well as the worldwide growing locavore movement focusing on the use of local food produce (i.e., "terroir"). This gave rise to what has come to be known as phycogastronomy, which can be defined as the art (and science) of cooking with seaweeds, taking advantage of their healthy and nutritious nature and organoleptic properties (i.e., visual appeal, colours, shapes, taste, flavour and mouthfeel). Today, phycogastronomy is slowly catching on in other casual or mid-range restaurants as well as in home cooking. The multi award-winning book "Those curious and delicious seaweeds, a fascinating voyage from biology to gastronomy" covers this exciting world of human consumption of seaweeds from a historical perspective, including many "phycoanecdotes as a side dish".

### **KEYNOTE**

#### When algae go public: revenge of the margins?

#### Levain, A.

UMR 6308 AMURE, CNRS, France alix.levain@univ-brest.fr

From the multiple but generally discreet relationships linking human populations with algae (seen as a general group of vegetative non-human beings inhabiting, adorning and hopefully stranding on sea bottoms, rocks and shores), emerge on the contemporary period new forms of problematization of algae's presence. In other words, algae tend to acquire the status of a public figure; many of them leave local margins to burst in the political space and gain *secondary* social visibility, overflowing their objective and material presence.

This presentation will build on the accounts of several ethnographic inquiries on algae's politicization and a recent interdisciplinary assessment led by social scientists on coastal eutrophication, to explore several fronts and arenas where social scientists document the conflictual nature of these bursts. Their works all exemplify how gust and disgust, praise and demonization, existential anguishes associated with environmental changes, and promises of health, nurturing, and blue economic expansion, come to terms when dealing with the socialization of algae.

While contributing to form a "public", in the sense of organized, self-conscious groups of people affected by social arrangements they are not associated with and claiming for political recognition, political struggles around algae's *right* place and behavior are a powerful driver of the emergence of algae as a popular culture singular contemporary object. The presentation will allow discovering and discussing some of the intriguing cultural representation they give birth to.

### **KEYNOTE**

### The importance with outreach in order to promote interest and use of Seaweeds in a Swedish and European context

#### Gröndahl, F.

Department of Sustainable Development, Environmental Science and Engineering (SEED), KTH, 100 44 Stockholm Sweden fgro@kth.se

During 2014 a new five-year strategical project for a Swedish biobased economy was launched. The project "SEAFARM" wanted to test if seaweed cultivation in combination with a biorefinery concept was possible on the Swedish Skagerrak coast. The project had a very holistic approach looking in to the cultivation, storage/preservation, biorefinery for food, feed and biomaterial, energy production (ethanol and biogas) and fertilizers. One of the most important parts of the project was also to evaluate the sustainability aspect in each steep of the process. The results of the project were very successful with very good seaweeds yields from the nutrient rich Skagerrak water and new interesting and innovative products based on seaweeds where demonstrated. The SEAFARM project also had the ambition to enhance the Swedish seaweed market that was very insignificant, so the project also started a number of activities to raise the interest and acceptance for seaweed cultivation among the public in Sweden. A small cookbook with information about seaweeds and some nice recipes was launched in collaboration with influencers (cooks) and the project partners was very active in different forms of outreach both in Sweden but also in the EU, demonstrating both food and biobased materials. A collaboration with Aalto University started with the "Department of Seaweeds" where we created creative workshops focusing on art, textiles and food based on seaweeds. The project also got a very good media coverage with articles, radio and television productions all around seaweed and the interest for seaweeds increased very fast in Sweden and eventually the SEAFARM project with the help of follow up projects lead to a number of new fast growing commercial seaweed companies in Sweden. The current presentation will tell the story on how a new seaweed-based biomass only 8 years after the first research trials now is the start of a new biobased industry in Sweden.

#### One-on-one and small group mentoring in phycology

#### Norici, A.

Laboratory of Algal and Plant Physiology, Dipartimento Scienze della Vita e dell'Ambiente, Università Politecnica delle Marche a.norici@univpm.it

Looking at most striking and promising research of recent times the task of understanding Life is approached by profiling cells at the individual level. In the same way as single-cell analysis, we may embrace single-student training and mentoring to move into a post-pandemic vulnerable world which urgently calls for young generations with critical thinking and analytical skills, creativity, and problem-solving abilities. Thus, teaching may be approached with similar rigour, curiosity, and appreciation for the scientific method that we experience in the laboratory.

I will show a few cases of active learning in interdisciplinary team groups as well as in mentor mentee relationship or in peer mentoring (as found in a lab): i.e. blended intensive program in Erasmus; advising business idea in contamination labs, supervising thesis; writing letters of recommendation. Phycology is a fundamental discipline which can be applied in actions to mitigate human-caused climate change and to plan and implement feasible and effective adaptation options; indeed, phycology becomes the stepping stone to a rediscovered sense of wonder, the starting point for a transformational learning experience having deep and positive impact on students' engagement with personal growth, academic habits and attitudes, student initiative and entrepreneurship.

I believe opportunities of one-on-one and small group advising are effective in challenging students to inquiry and to apply what they are learning to change the world, in building community around teaching and learning, in sharing evidence-based practices to educate citizens and citizen leaders.

### Seaweed hype, social media education to sway the opinion of the general public

#### Kraan, S.

The Seaweed Company, Headford, Ireland stefan.kraan@theseaweedcompany.com

The Oceans are of crucial importance for human survival. Nevertheless, global warming and overfishing threaten Earth's largest habitat, not to mention the plastic crisis. Phytoplankton responsible for about 50% of the oxygen in our atmosphere, sequesters carbon by sinking into the deep, is an important actor in Earth's climate system. Lately, macroalgae have entered the mix, with wild promises of farming, upscaling, and climate solutions by sequestering  $CO_2$ . Indeed, seaweed cultivation can capture more than what a rain forest will do, making it a good candidate for carbon capture. But carbon capture is not sequestering, it just moves  $CO_2$  from one source to the next till it gets released again. Sinking seaweed to the deep sea? We have little understanding of what actually will happen but many claim that this is earth's solution. Recent discovery of deep warm water currents indicate that all seems not as we think it is. A lot of factually incorrect statements have been used lately and large amount of money invested on false promises. No standard equation exists for carbon offsetting using seaweed; more data and scientific insight is needed. If we scale up cultivation, why not use the biomass for sustainable food

production or a sustainable polysaccharide industry, and become part of the solution of our plastics problem creating biodegradable single use plastics. In this presentation we will discuss the hype and hear many examples from the past which can be lessons for the future.

### KEYNOTE

### What can the Gulf of Mexico and Panama tell us about education and outreach?

Fredericq, S.<sup>1</sup>, Wysor, B.<sup>2</sup>, Freshwater, D.W.<sup>3</sup>, Krayesky-Self, S.<sup>1</sup>, Collin, R.<sup>4</sup>, Sauvage, T.<sup>5</sup>, Richards, J.<sup>1</sup>, Gabriel, D.<sup>5</sup>, Arakaki, N.<sup>7</sup>, Camacho, O<sup>8</sup>., Cho, T.O.<sup>9</sup>, Won, B.Y.<sup>9</sup>, Ehrenhaus, C.<sup>1</sup>, Venera-Ponton, D.<sup>4</sup>, Kittle, R.<sup>1</sup>, Krayesky, D.<sup>10</sup>, Gurgel, C.F.<sup>11</sup>, Schmidt, Wm.<sup>1</sup>

\*slf9209@louisiana.edu

A series of research grants funded by the National Science Foundation involved a major component about education and outreach as it pertained to marine algal diversity. These included comprehensive studies into 1) the diversity of the deep bank marine algae in the Gulf of Mexico (NSF Biodiversity Surveys and Inventories program) and the discovery of unsuspected eukaryotic life inhabiting rhodolith-forming coralline algae (NSF DEB), 2) monographic research (NSF PEET), 3) advanced tropical phycology with the integration of modern and traditional techniques in the study of tropical algae of Panama (NSF PASI), among others.

Education was very closely tied to outreach. Outreach included 1) field experience for undergraduate and graduate students in coastal and offshore subtropical and tropical marine environments; 2) high school teacher education focused on the inclusion of algae into high school biology curricula, 3) on-site high school and grade school visits with students' handson interaction with marine algae and algal products, 4) mentored research for undergraduate students in molecular phylogenetics and algal morphology that included research presentation at multiple avenues and publications.

Algae are wonderful organisms to convey fundamental biological concepts that can serve as a foundation to many career pathways.

### POSTERS

#### SYMPOSIUM 01 "Taxonomy and Systematics"

### Brijuni National Park (Adriatic Sea, Croatia) phytoplankton biodiversity assessment

Baričević, A.<sup>1\*</sup>, Smodlaka Tanković, M.<sup>1</sup>, Pfannkuchen, D.M.<sup>1</sup>, Grižančić, L.<sup>1</sup>, Vlašiček, I.<sup>1</sup>, Knjaz, M.<sup>1</sup>, Hervat, M.<sup>2</sup> & Pfannkuchen, M.<sup>1</sup>

<sup>1</sup>Ruder Boskovic Institute, Center for Marine Research, Laboratory for Evolutionary Ecology, Croatia <sup>2</sup>Brijuni National Park, Croatia \*ana.baricevic@cim.irb.hr

Brijuni National Park (Brijuni NP) is a marine protected area (MPA) consisting of 14 islands, islets and reefs in the northern Adriatic Sea. There is a significant lack of data on marine biodiversity in this unique ecosystem. Biodiversity monitoring is the most important measure for assessing the state of an ecosystem and a prerequisite for planning the conservation of protected areas. Over a period of one year (November 2020 - December 2021), monthly sampling was carried out at five stations representing marine coastal zones with different characteristics (seabed type, benthic community, physical parameters). Diversity, abundance and succession of the Brijuni NP phytoplankton community were described using the highthroughput molecular method of metabarcoding (18S and rbcL barcodes), supported by taxonomic expertise from microscopy. The phytoplankton community was dominated by dinoflagellates and diatoms throughout the year (in terms of abundance and taxa richness). There was a significant effect of sampling time on diatom community composition, but annual succession was represented by two instead of the usual four seasonal changes. During the year, representative taxa of other important phytoplankton divisions (Haptophyta, Cryptophyta, Chlorophyta) were also successfully identified, but they contributed less than 10 % to the total phytoplankton abundance and had a lower taxa richness than diatoms and dinoflagellates. Effects of environmental parameters on phytoplankton community distribution patterns were recorded. The results of this study represent the first comprehensive description of the Brijuni NP phytoplankton community and serve as a valuable benchmarking study for the future research and monitoring of this MPA.

#### Which microscope to take for field work

Colin, S.

MPI for Biology Tübingen, Germany \*sebastien.colin@tuebingen.mpg.de

The study of macro- or micro-algae often requires the collection of samples in the field. In general, sampling is carried out without the possibility of micro-observation. In the best of cases, the location allows the study of live specimens with scientific equipment in a laboratory close enough to the sampling site. However, in remote locations, samples are usually preserved in the field to delay their exploitation. No preservation method is perfect and this can affect the quality of the analysis.

Access to digital microscopy in the field to document or confirm the quality of samples is becoming easier. I will present and compare several transportable microscopes, commercial or open source, that can be used in the field for image acquisition, and potentially, quantitative analysis.

# *Ulva pilifera* (Ulvales, Chlorophyta) from Montenegro – application of X-ray fluorescence techniques in determining the habitat preferences

Czerwik-Marcinkowska, J.1\*, Piwowarczyk, R.1 & Wojciechowska, R.2

<sup>1</sup>Institute of Biology, Jan Kochanowski University, Poland

<sup>2</sup>Department of Geobotany and Landscape Planning, Nicolaus Copernicus Uni9versity, Poland \*joanna.czerwik-marcinkowska@ujk.edu.pl

A green macroalgae was collected in Montenegro from four new sites (freshwater and marine: a river, a ditch, the Milet Canal, and on the rocky shore of the Adriatic Sea. A detailed morphology, autecology, and molecular studies allowed to identify the *Ulva* population as *Ulva pilifera*.

Using total reflection X-ray fluorescence (TXRF) and wavelength dispersive X\_ray fluorescence (WDXRF) techniques it assessed the elemental composition of their thalli and its influence to colonise new habitats. In elemental analysis, 25 elements were determined, i.e., Al, As, Ba, Br, Ca, Cl, Cr, Cu, Fe, Hf, I, K, Mg, Mn, Na, Ni, P, Pb, Rb, S, Si, Sr, Ti, V, and Zn. The highest elemental concentrations were found for Ca = 16.3% (using WDXRF) and for Sr = 292 ppm (using TXRF) in the *Ulva* thalli. The analysis of trace elements showed that the metal content in *Ulva* thalli is correlated with the trace elements in water and sediments.

### A study on the variation of nutritional profiles of seaweeds along the Senegalese coast

Fricke, A.<sup>1\*</sup>, Bousso, N.C.<sup>2,3,4</sup>, Schreiner, M.<sup>1</sup>, Ndiaye, W.<sup>4</sup>, Brehmer, P.<sup>3</sup>, Diop, M.S.<sup>3,4,5,6</sup>, Kane, C.<sup>2</sup>, Quack, B.<sup>6</sup> & Baldermann, S.<sup>1,7</sup>

<sup>1</sup>*IGZ*, Groβbeeren, Germany <sup>2</sup>*Ecole Supérieure Polytechnique (ESP), UCAD, BP 5085, Dakar, Sénégal* <sup>3</sup>*IRD, Univ Brest, CNRS, Ifremer, Lemar, SRFC, Dakar, Sénégal* <sup>4</sup>*ISRA, CRODT, Dakar, Senegal* <sup>5</sup>*UCAD, FST, Dakar, Senegal* <sup>6</sup>*GEOMAR, Kiel, Germany* <sup>7</sup>*University of Bayreuth, Germany* \*fricke@igzev.de

Driven by the need for alternative food sources, there is a growing global interest in edible macroalgae ("seaweeds"). Rich in protein, they also provide bioactive compounds with health promoting benefits. Nutritional compounds like carotenoids, chlorophylls and fatty acids play a key role in human nutrition and are used as coloring agents in the food and related industries (e.g. pharmacy). With a coastline of about 700 km, Senegal harbors a diverse maritime life, including a huge variety of natural growing seaweeds. Despite the known natural high diversity, there have been only few attempts to study this natural resource. With the aim to provide an overview on the distribution of nutritional valuable seaweeds, the CLIMALG project conducted two field missions in 2021 (cold season) and 2022 (hot wet season), collecting seaweeds along the Senegalese coast (14° 41' 37.32" N; -17° 26' 38.62" W). From the total of 675 samples taken 69 were chosen to study the photosynthetic pigment and fatty acid composition in relation to species identity and location. Right after collection samples were frozen, lyophilized, transferred and analyzed by HPLC-MS for their photosynthetic pigments profiles and by

GC-MS to determine their fatty acid composition. The identification and quantification of 13 fatty acids (3 PUFAs, 2 MUFAs, 8 SFAs), 9 carotenoids, 3 chlorophylls and their isomers was done by using authentic reference compounds. Here we show the first results of the study reflecting the diverse metabolite profiles, which shall provide us with a better understanding on the nutritional value of Senegalese seaweeds.

### *Dictyota psychedelica* sp. nov., a new prostrate member of the genus in the Caribbean Sea

Corrales, M.B.<sup>1</sup> & Gavio, B.<sup>2\*</sup>

<sup>1</sup>Universidad Nacional de Colombia, sede Caribe, San Andrés isla, Colombia <sup>2</sup>Universidad Nacional de Colombia, sede Bogotá, Bogotá, Colombia \*bgavio@unal.edu.co

The genus *Dictyota* (Phaeophyceae) is a common member of benthic communities, and its biogeographic affinity is mainly tropical to warm temperate. As other members of the order Dictyotales, the genus has suffered a considerable rearrangement of its taxonomy in the past fifteen years, with the description of new species based on molecular and morphological analyses and the delimitation of oncebelieved cosmopolitan species.

In the tropical western Atlantic there are twenty-one species of *Dictyota*; of these, thirteen are recorded for the *Seaflower* Biosphere Reserve, in the southwestern Caribbean Sea. Only two of the registered species (*Dictyota humifusa* and *D. friabilis*) have a clear prostrate habit. Due to the presence of secondary metabolites, their ability to propagate through fragmentation and the demise of coral communities, members of the genus are among the most widespread and abundant components of benthic communities in the Caribbean Sea and the Seaflower Biosphere Reserve is no exception.

These species have a variety of growth forms, since they may be completely erect, decumbent, prostrate with non-attached parts, or firmly adherent to the substrate. They may form dense monospecific mats or live intermixed with other species of the genus. Some species show structural color (=iridescence).

During recent surveys on marine macroalgae at several localities of the Seaflower Reserve, a diversity previously overlooked of members of the genus *Dictyota* has been observed. Among the species collected, an undescribed prostrate taxon with peculiar structural color was discovered, and it is hereby characterized.

## Cryptic diversity in the Prasiolaceae (Prasiolales, Trebouxiophyceae, Chlorophyta)

#### Heesch, S.

Applied Ecology & Phycology, University of Rostock, Germany svenja.heesch@uni-rostock.de

The Prasiolaceae is a small family of mainly aero-terrestrial green algae that is well known for the preference of its members for high-nutrient environments. Some species are found at coastal sites in and near seabird colonies, while others can be observed in nutrient-enriched urban habitats. In central Europe they commonly occur, for example, where male mammals urinate at the base of trees or behind pubs. But members can also be found in more unusual places: A recent expedition to the Arctic, for instance, revealed the presence of undescribed microscopic Prasiolaceae on biogenic hard substrate. This study clearly demonstrates that an integrated approach combining classical isolation and cultivation methods

with molecular genetic techniques is crucial for the discovery and description especially of minute algae. Moreover, it suggests that terrestrial habitats remain a major repository of unknown algal diversity.

### Diversity of the freshwater macroalgae from Guadeloupe, French Lesser Antilles

Helias, M.<sup>1\*</sup>, Vis, M.L.<sup>2</sup>, Bidault, A.<sup>1</sup>, Necchi Jr., O.<sup>3</sup>, Labeille, M.<sup>4</sup> & Burel, T.<sup>1</sup>

<sup>1</sup>Univ Brest, CNRS, IRD, Ifremer, LEMAR, F-29280 Plouzané, Brittany, France

<sup>2</sup>Country Department of Environmental and Plant Biology, Ohio University, Athens, Ohio 45701, USA
 <sup>3</sup>Zoology and Botany Department, São Paulo State University, Rua Cristóvão Colombo, 2265-15054-000 São José do Rio Preto, Brazil
 <sup>4</sup>Sentinelle Lab, 97125 Bouillante, France

\* mathieu.helias26@gmail.com

Freshwater macroalgae are studied worldwide, but their diversity and distribution are still poorly known. This study, focusing on Guadeloupe island freshwater habitats aims to 1) provide an up-to-date list of species found in Guadeloupe streams, 2) investigate their distribution using environmental variables and 3) reassess the taxonomic status of the rare and potentially endemic *Batrachospermum pulchrum*. In early February 2023, a total of 49 sites were sampled. Stream sections, 20-meters in length, were examined, the percentage of substrate cover was recorded, and water parameters (pH, conductivity, temperature) were measured. Macroalgal samples were identified using morphological characters and DNA sequence data. Mean number of species per stream segments was  $2.4 \pm 1.4$  (1 - 6), similarly to other tropical areas (*e.g.* Hawaii). Morphological identification revealed a total of 27 taxa, which is relatively low compared to nearby regions. Identified taxa consisted of 8

Rhodophyta, 6 Chlorophyta, 5 Charophyta, 7 Cyanobacteria, and 1 Ochrophyta. *Hildenbrandia angolensis*, *Spirogyra* sp. and *Bostrychia radicans* were the most common species, found at 25, 18 and 10 localities, respectively. The red algal order Batrachospermales only occurred in 6 sites. The rarely recorded *B. pulchrum*, described from Guadeloupe, was collected in 2 localities. *Catenella* sp. was found for the first time in freshwater at two localities. Some species showed more affinity towards ponds, ravines or rivers. This study emphasizes the diversity and distribution of freshwater macroalgae in Guadeloupe, as well as the occurrence of rare species. It provides a foundation for broader freshwater surveys in the Caribbean region.

#### A study of marine benthic diatoms from Ulleungdo and Dokdo, Korea

Yangrae Rho, Y. & Park, J.\*

Ocean Science and Technology School, Korea Maritime and Ocean University, Busan, South Korea, and Korea Institute of Ocean Science and Technology, Busan, South Korea.

In the present study, marine benthic diatoms from remote islands of Korea viz. Ulleungdo and Dokdo have been observed and identified with Light Microscope and Scanning Electron Microscope. Total of six samples have been collected, five from Ulleungdo and one from Dokdo. Four of the Ulleungdo samples were from the subtidal zone and one sample was from tidal zone, while the Dokdo sample was collected from the tidal zone. A total of 249 species from 70 genera has been identified with *Amphora*, *Diploneis*, and *Cocconeis* being the most dominant genera. Among these 30 species have been newly observed from the Korean waters. A number of prospective new diatom taxa have also been observed including a possible new genus. In the meantime, occurrences of rare diatoms such as *Colliculoamphora* 

*minima*, *Cuneolus skvortzowii*, *Cybellonitzschia banzuensis*, *Tripterion societatis* and *Tryblioptychus hainanesis* were found. The result of the present study has highlighted the unique biodiversity of Korean remote islands particularly in terms of diatom diversities.

# Coralline algae (Rhodophyta, Corallinales) early colonizers of the littoral lava flows of the recent volcanic eruption of La Palma (Canary Islands)

Peña, V.<sup>1</sup>\*, Afonso-Carrillo, J.<sup>2</sup>, Álvarez-Canali, D.<sup>2</sup>, Liulea, S.<sup>2</sup>, Rancel-Rodríguez, N.M.<sup>2</sup>, Reyes, J.<sup>2</sup>, Sansón, M.<sup>2</sup> & San Gil, C.<sup>2</sup>

<sup>1</sup>BioCost Research Group, Department of Biology, University of A Coruña, Spain <sup>2</sup>Departamento de Botánica, Ecología y Fisiología Vegetal, Universidad de La Laguna, Tenerife, Canary Islands, Spain \*v.pena@udc.es

The eruption of the Tajogaite volcano (La Palma, Canary Islands) occurred between September 19 and December 13, 2021. The lava flowed into the sea on the western shore of La Palma and a new rocky shore was formed. Once the eruption ended and the safety protocols allowed it (February 2022), several bimonthly samplings were carried out in the intertidal and shallow subtidal in order to evaluate the contribution of coralline algae in the early stages of colonization of these new substrates. The identification was carried out by morpho-anatomical and molecular studies. Just two months after the end of the eruption, coralline algae had already settled on the surface of volcanic rocks initially colonized by bacteria and diatoms. The first multicellular colonizers were annual ephemeral minute and thin species of the genus *Hydrolithon. Hydrolithon* crusts bearing a large number of minute triangular vegetative propagules were very common, suggesting that vegetative reproduction by specialized propagules may play a relevant role in these early stages. These *Hydrolithon* were later covered and replaced by other slightly thicker non-fertile crusts. Both small confluent crusts of *Porolithon* and crusts that ended up forming *Corallina*-type erect geniculate branches became the coralline algae dominant. Trichocytes, isolated or in dense groups, were very obvious in all coralline algae that took part in these early stages of colonization of the new volcanic substrates.

# Revisiting the systematics of the *Grateloupia*-complex (Halymeniales, Rhodophyta) based on cystocarp development and molecular phylogeny

Rodríguez-Prieto, C.<sup>1\*</sup>, De Clerck, O.<sup>2</sup>, Huisman, J.M.<sup>3</sup>, Leliaert, F.<sup>4</sup> & Lin, S.-M.<sup>5</sup>

<sup>1</sup>Faculty of Sciences, University of Girona, Spain

<sup>2</sup>*Phycology Research Group and Centre for Molecular Phylogenetics and Evolution, Ghent University, Ghent, Belgium* 

<sup>3</sup>Western Australian Herbarium, Biodiversity and Conservation Science, Bentley Delivery Centre, Western Australia

<sup>4</sup>Meise Botanic Garden, Meise, Belgium

<sup>5</sup>Institute of Marine Biology, National Taiwan Ocean University, Taiwan, R.O.C.

\*conxi-rodríguez@udg.edu

The *Grateloupia*-complex in the red algal order Halymeniales contains ca. 116 species placed in seven genera. Most were originally placed in *Grateloupia* C. Agardh, one of the largest genera within the Rhodophyta. However, the current classification remains controversial. Recent studies that combined DNA sequence analyses (including chloroplast phylogenomics), and data on pre- and post-fertilization development provide substantial clues for resolving the intergeneric relationships of this complex. In particular, the architecture of the auxiliary cell ampullae (= the female reproductive structure that will receive the diploid nucleus resulting from karyogamy and gives rise to the carposporophyte generation) and its behavior before and after diploidization are critical for delimiting genera. In this presentation, we re-examine the generic concepts of *Phyllymenia* J. Agardh, *Prionitis* J. Agardh, *Dermocorynus* P. Crouan & H. Crouan, and *Mariaramirezia* M.S. Calderon, G.H. Boo, A. Mansilla & S.M. Boo and highlight critical pre- and post-diploidization stages in order to resolve the systematics of the Halymeniales, based on a selection of representative species.

### Contribution to the taxonomy of *Sargassum* with specimens from French West Indies and south of the Madagascar coasts

Rousseau, F.<sup>1</sup>\*, Marani, G.<sup>1</sup>, Blanfuné, A.<sup>2</sup>, Pena-Martin, C.<sup>3</sup>, Robledo, D.<sup>4</sup>, Le Gall, L.<sup>1</sup>& Thibaut, T.<sup>2</sup>

<sup>1</sup>Institut de Systématique, Evolution, Biodiversité (ISYEB), France <sup>2</sup>Institut Méditerranéen d'Océanologie (MIO - UMR 7294), France <sup>3</sup>Universidad de Alicante <sup>4</sup>Department of Marine Resources, CINVESTAV-IPN \*Florence.rousseau@mnhn.fr

The genus *Sargassum*, is the most diverse genus among brown algae with 361 species currently accepted. Although the news highlights the abundant stranding events of two species on the coasts of the West Indies and Florida with dramatic economic consequences, the other species are all benthic. While the genus is well defined, species delineation based on morphological characters remains controversial with many species complexes where phenotypic plasticity prevents the identification of diagnostic traits. To better evaluate species boundaries and their actual distributions, more studies coupling an integrative approach including molecular characters with a comprehensive sampling are needed. We studied more than 200 specimens collected during the expeditions "Our Planet Reviewed" along the coasts of Martinique, Guadeloupe (French Antilles) and southern Madagascar. The sequencing of several molecular markers (ITS, cox3, rbcL spacer...) provides the opportunity to reevaluate the species richness of the investigated regions and to contribute to a better understanding of the species distribution and taxonomy of the genus *Sargassum*.

### The application of population genomics in the taxonomy of cyanobacteria

Dvořák, P.<sup>1</sup>, Jahodářová, E.<sup>1</sup>, Stanojković, A.<sup>1</sup>, Skoupý, S.<sup>1\*</sup> & Casamatta, D.A.<sup>2</sup>

 <sup>1</sup>Palacký University Olomouc, Faculty of Science, Department of Botany, Šlechtitelů 27, 78371 Olomouc, Czech Republic
 <sup>2</sup>University of North Florida, College of Arts & Sciences, Department of Biology, 1 UNF Drive, FL 32224-7699, Jacksonville, USA
 \*svatopluk.skoupy01@upol.cz The emergence of cyanobacterial species (speciation) is propelled by mutation, selection, and geographical and ecological isolation. Furthermore, homologous and non-homologous recombination entangle evolutionary relationships among the lineages. These factors potentially play a significant role in the species delimitation and, therefore, in taxonomy. The use of several neutral markers or small sampling can provide only limited insight into the cyanobacterial speciation. With the availability of cheap high-throughput sequencing, we can investigate whole-genome diversity and population level divergence in space and time applying the population genomics. We present a framework of population genomics methods and approaches, and their implementation to the taxonomy of cyanobacteria. Ultimately, that will allow to make the taxonomic decisions as close to the nature as possible.

#### A genomic approach towards resolving the taxonomy and phylogenetic relationships of historic stonewort (Charophyta) collections

Wilbraham, J., Price, B., Hall, A., White, O. & Brodie, J.

Natural History Museum, London, UK

The charophytes (stoneworts) are a group of evolutionary, ecological and conservation significance. They are ancestral to the land plants, indicators of water quality and under threat from degradation and loss of water bodies. The algal herbarium at the Natural History Museum, London holds an internationally significant collection of c. 20,000 charophyte specimens, including vouchers for all British and Irish species/varieties. Unlocking the identity of these internationally important historic collections using a genomic approach will deliver a pivotal step in resolving long standing taxonomic issues and towards resolving relationships of British and Irish charophytes with those in the rest of the world. To achieve this goal, we are undertaking a study to develop a genome skimming protocol for use with the charophyte herbarium specimens. To develop this approach, an initial set of specimens includes representative samples from all five charophyte genera, Chara, Nitella, Nitellopsis, Lamprothamnium and Tolypella, known from Britain and Ireland and includes specimens of 20 charophyte species. An initial trial has yielded DNA from 37 specimens including from material collected in the 1890s, with recovery of the plastid genome. The results of this study will be a re-evaluation of historical and contemporary taxon diversity in Britain and Ireland and provide phylogenetic context to understand relationships with charophytes from other parts of the world. The protocols developed in this project will potentially provide a standard approach that can be adopted for use with historical herbarium specimens.

#### Diversity and distribution of desmids in Britain and Ireland

Wilbraham, J. & John, D.M.

Natural History Museum, London, UK

Freshwaters are amongst the most threatened habitats in the world. The conservation of biodiversity associated with these water bodies has focused on the larger animal and plant species, however, microscopic organisms, including algae such as desmids (Chlorophyta) and diatoms (Bacillariophyta), play a fundamental role in these ecosystems and constitute rich assemblages that are equally at threat. Their conservation, however, poses many difficulties due to taxonomic impediments and lack of knowledge of their ecology and distribution. Britain and Ireland is a particularly diverse region for

desmids with over 1,300 taxa recorded. However, the list of species recorded historically from these islands is in need of critical review. Checklists are an essential tool for understanding biodiversity, providing a robust framework for the consistent use of botanical names. Similarly, reliable datasets of species occurrence are vital to biodiversity research and conservation. To gain a better understanding of current desmid distribution patterns across these islands, we have digitised 50 years of biological recording data collated by desmid expert David Williamson. Building on that baseline, we are undertaking a critical review of all desmid taxa recorded from Britain and Ireland including from contemporary collections. This work is enabling the addition of new reports of taxa from the region and the removal of those which had been erroneously listed. It is also revealing that large numbers of desmids recorded in the older literature have not been rediscovered in the past 50 years, in some cases not since the early 1900s.

### DNA barcoding and chemical analysis of algae from Malta in the central Mediterranean

Zammit, G.<sup>1,2</sup>

<sup>1</sup>Laboratory of Applied Phycology, Centre for Molecular Medicine and Biobanking, University of Malta, Msida, MSD2080, Malta

<sup>2</sup>Microbiology Laboratory, Department of Biology, Faculty of Science, University of Malta, Msida, MSD2080, Malta

\*gabrielle.zammit@um.edu.mt

Until recently, the only scientific study focused on the macroalgal biodiversity from Maltese coastal waters was a checklist of algal species based exclusively on morphological data. Consequently, the marine environment around Malta contains overlooked algal biodiversity producing valuable metabolites with the potential to be used as a sustainable food source. Thus, recently, considerable effort has been made to describe the widespread cryptic algal diversity from a variety of habitats around the Maltese islands.

The research was carried out as part of a project titled 'Green Algae to Solve Emerging problems in food security' (GreASE), which involved the identification, sustainable growth and chemical analysis of suitable algal candidates for application in the food industry.

The project studied algal biodiversity via a multiphasic approach that included genetic and chemical analysis. Novel algal diversity has been recorded both from the land and sea, using DNA barcoding, coupled to culturing of evasive species of cyanobacteria, microalgae, as well as red, brown and green algae. The DNA barcodes included the SSU rRNA genes, the 5'-COI gene (*cox*1, cytochrome oxidase, subunit I - 5' end), the *rbcL* gene (ribulose bisphosphate carboxylase large chain), *rbcL-rbcS* spacer, *tuf*A and ITS1+5.8S+ ITS2. The algal biomass was chemically analysed and characterised by metabolic profiling for potential use as sustainable food and animal feed.

The project was financed by the Malta Council for Science & Technology (MCST), for and on behalf of the Foundation for Science and Technology, through the FUSION: R&I Research Excellence Programme'.

## SYMPOSIUM 02 "Biodiversity assessment and algal distribution in space and time"

#### AlgaeTraits.org: a trait database for seaweed species

#### AlgaeTraits editors

\*algaetraits@marinespecies.org

The analysis of biological and ecological traits has a long history in evolutionary and ecological research. However, trait data are often scattered and standardised terminology that transcends taxonomic and biogeographical context are generally missing. As part of the development of a global trait database of marine species, we collated trait information for European seaweeds and are working towards a global seaweed trait database.

So far, we collected 45,175 trait records for 21 biologically and ecologically relevant traits of seaweeds. This resulted in a trait database for 1,745 European seaweed species of which more than half (56%) of the records were documented at the species level, while the remaining 44% was documented at a higher taxonomic level and subsequently inherited at lower levels. The trait database for European seaweeds will serve as a foundation for future research on diversity and evolution of seaweeds, and their responses to global changes. The data can be used to contribute to developing detailed trait-based ecosystem models, and can be an important tool to inform marine conservation policies. The data is publicly accessible through the AlgaeTraits portal, algaetraits.org.

### The occurence of new *Pseudo-nitzschia* speceis: *P. brasiliana*, *P. hasleana* and *P. linea* in the Adriatic Sea

Arapov, J.\*, Tomašević, T., Bonačić, T., Pejković, M., Bužančić, M., Mandić, J. & Bušelić, I.

Institute of Oceanography and Fisheries, Croatia \*arapov@izor.hr

Marine diatoms of the genus *Pseudo-nitzschia* are a common component of the phytoplankton community in the Adriatic Sea. Due to the existence of cryptic species, the taxonomic composition is still quite unknown along the eastern coast. In general, half of the *Pseudonitzschia* species described today have been shown to produce the neurotoxin domoic acid (DA), causing Amnesic Shellfish Poisoning (ASP).

Herein, we present the preliminary results of *Pseudo-nitzschia* species composition studied from February 2022-2023 in four aquaculture areas, coupling morphological and molecular methods. The studied areas differ in environmental conditions, mainly due to the force of freshwater input. Monthly taken plankton net samples (integrated depth surface-5m) were analysed by field emission scanning electron microscope. Twelve *Pseudo-nitzschia* species were morphologically determined, and among them, *P. brasiliana*, *P. linea* and *P. hasleana* were recorded for the first time in the Adriatic Sea. Species *P. brasiliana* and *P. hasleana* occurred only in Kaštela Bay, while *P. linea* was found in all areas. In addition, the species *P. hasleana* was successfully isolated and confirmed according to molecular data of LSU and ITS gene sequence.

Although the species from *P. delicatissima* complex and *P. cf. pseudodelicatissima* were generally the most frequently occurring and occasionally prevailing in the assemblage, different seasonal patterns and species contributions were observed among the areas. The highest contribution of *P. calliantha* and *P. linea* in the *Pseudo-nitzschia* assemblage was found in the northern area, while *P. galaxiae* was prevailing in the southern part.

#### Akashiwo sanguinea - 10 years in Wales

Bear, B.\* & Almeida, N.

*Cefas, UK* \*beth.bear@cefas.gov.uk

We investigated the occurrences of *Akashiwo sanguinea* along the Welsh coastline from ~2013. *A. sanguinea*, although not toxic, is considered a nuisance species and is on the UK's invasive species list. It has been noted in routine monitoring for both Environment Agency/Natural Resource Wales Water Framework Directive assessments and Food Standards Agency Marine Biotoxin Water Sampling Programmes in English and Welsh waters, and anecdotally there has been a spatial spread since 2015. It has been suggested that it was originally brought to the UK in ballast waters on ships and notably a population was sustained in an enclosed dock in South Wales. This study investigates whether this species has spread along the Welsh coastline both temporally and spatially.

This study has been conducted on available data from the assessments for the Welsh Coastline. Data is also available from England's coastline; this does not form part of this study but may be included in a more extensive future one. Data is presented as presence only.

Samples were collected at least monthly and preserved with acidified Lugol's iodine at collection. They were analysed using the Utermöhl method and identification was under light microscopy only.

This study does not consider the driving factors behind any spread in the occurrences – combining this data with sea temperature or weather data may give an indication of likely future spread and a complementary study looking at this will be considered.

### Chlorophyll-*f*-producing cyanobacterial communities in selected karst caves of Northern Italy

Bellamoli, F.<sup>1\*</sup>, Latella, L.<sup>2</sup>, Cantonati, M.<sup>3</sup>, Calgaro, M.<sup>1</sup>, Pivato, M.<sup>1</sup>, Laiti, C.<sup>3</sup>, Cazzaniga, S.<sup>1</sup>, Vitulo, N.<sup>1</sup> & Ballottari, M.<sup>1</sup>

<sup>1</sup>University of Verona, Department of Biotechnology, Strada le Grazie 15, Verona, 37134, Italy

<sup>2</sup>Museo di Storia Naturale of Verona, Department of Zoology, Lungadige Porta Vittoria 9, Verona, 37129, Italy

<sup>3</sup>University of Bologna, Department of Biological, Geological, and Environmental Sciences, Via Selmi 3, Bologna, 40126, Italy

\*francesco.bellamoli@gmail.com

Caves are seldom considered an environment where phototrophic organisms can grow, but they can be found in most parts reached by direct or indirect sunlight. Among phototrophs, cyanobacteria have the ability to adapt to harsh environmental conditions, such as dimly illuminated cave sections with low temperatures. Depending on light conditions, through a remodeling of the photosynthetic apparatus (FaRLiP) and by synthesizing alternative chlorophylls (chl *d* and/or *f*), cyanobacteria can also utilize farred light to drive photosynthetic processes. Although far-red absorbing chlorophylls have been described

in several cyanobacterial species, their occurrence and distribution, in terrestrial and especially in cave habitats, is still scarcely documented.

In this study, we examined aerophytic epilithic cyanobacterial populations and associated prokaryotic communities in three karst caves of Northern Italy. In particular, for each sampling site we analyzed whole pigment content by HPLC and on individual cells by LCSM, thus identifying chlorophyll-*f*-containing samples. We also performed metabarcoding analyses on 16S rRNA V3-V4 and ITS2 regions to determine the composition of prokaryotic and associated eukaryotic communities.

Subsequent analyses revealed complex communities with strong taxonomic differences in very small distances on a spatial scale, with light availability as the main driver. We determined possible cyanobacterial taxa associated with chlorophyll *f* production, highlighting the widespread diffusion of chlorophyll *f*-producing cyanobacteria in those ecological niches.

### Diversity and toxigenic characteristics of cultured *Pseudo-nitzschia* species from East Adriatic Sea

Bonačić, T.<sup>1\*</sup>, Tomašević, T.<sup>1</sup>, Pejković, M.<sup>1</sup>, Bušelić, I.<sup>1</sup>, Lepen Pleić, I.<sup>1</sup>, Ujević, I.<sup>1</sup>, Bulić, A.<sup>1</sup>, Roje Busato, R.<sup>1</sup>, Penna, A.<sup>2,3</sup>, Casabianca, S.<sup>2</sup> & Arapov, J.<sup>1</sup>

<sup>1</sup>Institute of Oceanography and Fisheries, Laboratory of Plankton and Shellfish Toxicity, Šetalište I. Meštrovića 63, 21000 Split, Croatia

<sup>2</sup>University of Urbino, Department of Biomolecular Sciences, Campus E. Mattei, 61029 Urbino, Italy <sup>3</sup>CoNISMa, National Inter-University Consortium for Marine Sciences, 00196 Roma, Italy

\*tinabonacic@gmail.com

The *Pseudo-nitzschia* species are cosmopolitan diatoms, which are basal functional group in the food web as they are primary producers. In our study, diversity was observed throught morphological and molecular analysis of cell cultures. Samples were collected monthly, from February 2022 to February 2023 on four locations: Kaštela and Malostonski bay, Šibenik and Velebit channel. To explore phylogenetic diversity and toxigenic characteristics cell cultures were established. Isolation was done by glass micropipettes, cells were harvested in mid-exponential phase by centrifugation and genomic DNA was extracted using the DNA Plant DNeasy protocol. Isolated species were described by both scanning electron microscopy and molecular analyses based on internal transcribed spacer (ITS) and large subunit region (LSU) of ribosomal DNA.

By molecular analysis and morphological features several species have been identified: as follows: *P. allochrona, P. calliantha, P. delicatissima, P. galaxiae P. hasleana, P. mannii, P. multistriata, P. pseudodelicatissima, P. subfraudulenta.* Intraspecific morphological and molecular variability within the same species were seldom detected. A total of 63 cultures were analysed for the presence of DA (domoic acid) by tandem mass spectrometry coupled with liquid chromatographic separation (LC–MS/MS). Cultures were harvested for toxin analysis at four growth phases by filtration through GF/F filters (0.7  $\mu$ m) for DA analysis. Among the analysed strains, eleven of *P. pseudodelicatissima* from Kaštela Bay shown the ability to produce DA.

### DNA-sequencing-based assessment of seaweed biodiversity and distribution along the Senegalese coast

Diop, M.S.<sup>1,2,3,4</sup>\*, Weinberger, F.<sup>4</sup>, Ndiaye, W.N.<sup>2</sup>, Mbaye, M.S.<sup>1</sup>, Brehmer, P.<sup>3</sup>, Brakel, J.<sup>5</sup>, Diedhiou, F.<sup>2</sup>, Fricke, A.<sup>6</sup>, Quack, B.<sup>4</sup> & Bolchi, G.<sup>2</sup>

<sup>1</sup>Laboratoire de Botanique et Biodiversité (LBB), Département de Biologie Végétale, UCAD, BP 5005, Dakar-Fann, Senegal

<sup>2</sup>Centre de Recherches Océanographiques de Dakar Thiaroye (CRODT), ISRA B.P. 2241 Route des Hydrocarbures Dakar, Senegal

<sup>3</sup>Laboratoire des Sciences de l'Environnement Marin (LEMAR), IRD BP 1386 Route des Hydrocarbures Dakar, Senegal

<sup>4</sup>GEOMAR Helmholtz-Zentrum für Ozeanforschung Kiel (GEOMAR), Düsternbrooker Weg 20 24105 Kiel, Germany

<sup>5</sup>Helmholtz Institute for Functional Marine Biodiversity at the University of Oldenburg (HIFMB), Ammerländer Heerstraße 231 26129 Oldenburg, Germany

<sup>6</sup>Leibniz-Institut für Gemüse- und Zierpflanzenbau (IGZ), Theodor-Echtermeyer-Weg 1 14979 Großbeeren, Germany

\*mamiesouadoudiop@gmail.com

Despite their ecological importance in coastal environments and their economic potential macroalgae in Senegal and West Africa in general have so far been understudied. In particular, molecular biological verifications of species inventories are still largely lacking. To generate the missing baseline knowledge we collected 1129 macroalgae samples from various substrates at 24 coastal sites along the Senegalese coast. The samples were identified based on morphological traits using dichotomous keys, DNA was extracted and PCR amplifications targeting the rbcL (Rhodophyta), COI (Phaeophyta) or tufA (Chlorophyta) gene were realized. Overall, the DNA barcoding success was relatively low, as only 20% of all samples provided analyzable sequences. This was expected to be due to sample contamination with epi- and endophytes, and we therefore analyzed algal samples in a second step by ampliconmetabarcoding, targeting rbcL. This library was sequenced on Illumina MySeq.

Most of the obtained sequences represented taxa that are already included in Senegalese species inventories. However, some specimens were genetically most closely related with *Gracilaria isabellana* and *Coeloseira compressa*, which have not yet been reported from Senegal but already from Cabo Verde. Also, the presence of *Ulva ohnoi* was confirmed, which is an invasive species from Japan and has now apparently reached Senegal. Thus, our study confirms the presence of seaweed invaders, as well as of cryptic species in Senegal. It also provides insight into recent species distributions along the Senegalese coast and it establishes the underlying methodology for further application in Senegal and its West African sub-region.

### Modelling of the effect of kelp harvesting and herbivory on fragmented forests

Dupont, G.<sup>1,3</sup>\*, Génin, A.<sup>2,3</sup>, Sallen Cruz, C. & Wieters, E.<sup>3</sup>

<sup>1</sup>International Master of Science in Marine Biological Resources (IMBRSea), Ghent University, Ghent, Belgium.

<sup>2</sup>Copernicus Institute of Sustainable Development, Utrecht University, PO Box 80115, 3508 TC, Utrecht, The Netherlands.

<sup>3</sup>Estación Costera de Investigaciones Marinas and Millenium Nucleus for Ecology and Conservation of Temperate Mesophotic Reefs Ecosystems (NUTME), Pontificia Universidad Católica de Chile, Las Cruces, Chile.

\*guillaume.dupont@imbrsea.eu

Along the Chilean coastline, the increasing impacts of fisheries on the fragmentation of kelp forests has motivated the development of compromises between extraction and conservation. This has taken the form of a set of extraction guidelines determining the intensity and method used to extract kelp, e.g. not extracting kelp below densities of 1 indiv.m<sup>-2</sup>.

We used a stochastic cellular automaton model and parameterized it using in-situ experiments in continental Chile to investigate how local interactions (e.g. competition for light) determine the spatial dynamics of kelp forests at a medium scale (about 4 hectares). We simulated different levels of forest fragmentation (as measured by the perimeter/area ratio of kelp patches) and management guidelines to (i) understand how fragmentation affects the resilience of the forest to realistic levels of extraction, and (ii) whether management guidelines were successful at improving this resilience.

Results show that fragmentation plays a certain role on the resilience of the forest as well as herbivory intensity, with higher levels of fragmentation and herbivory compromising the ability of the forest to recover from disturbance. However, extraction guidelines successfully increased the range of herbivory pressures for which kelp could persist through time.

Our work implies that extraction guidelines do help to maintain higher resilience of kelp forests but may have to be adapted for forests with a high density of herbivores and for high fragmentation levels. Despite the logistical difficulties of measuring fragmentation empirically, it could be a valuable addition to national monitoring programs of kelp forests.

### Arsenic dynamics during stranding and degradation of holopelagic *Sargassum* spp. in the Caribbean region

Gobert, T.<sup>1</sup>\*, Connan, S.<sup>1</sup>, Châtelain, B.<sup>1</sup>, Guillot, T.<sup>2</sup>, Stiger-Pouvreau, V.<sup>1</sup> & Waeles, M.<sup>1</sup>

<sup>1</sup>Univ Brest, CNRS, IRD, Ifremer, LEMAR, F-29280. Plouzane, France <sup>2</sup>Unité BIODIVENV, Ifremer Martinique, 79 route de Pointe Fort, 97231 Le Robert, France \*Tristan.Gobert@univ-brest.fr

Massive strandings of holopelagic Sargassum spp., originally from the Sargasso Sea, have been recurring since 2011 throughout the Caribbean zone. Three morphotypes have been reported: S. natans VIII, S. natans I and S. fluitans III. One of the main concerns with these brown macroalgae is their high arsenic content which can be released into the environment during the stranding/degradation process, particularly in its toxic inorganic forms, and may be harmful to coastal ecosystems. Understanding the fate of arsenic during this phase is critical for developing effective strategies to manage these strandings. Sampling and in situ coastal experiments have been performed in Martinique and Guadeloupe to study this degradation. First, samples have been collected along a gradient from nearshore to stranding, representing the different stages of the degradation process. The results indicate that these algae contain an average of  $100 \pm 13$  (range of 84-124) mg.kg<sup>-1</sup> DW of arsenic near shore. For the bay and stranding stations the arsenic concentrations show variability reflecting the difference in degradation state (range 27-140 mg.kg<sup>-1</sup> DW). Mesocosm experiments provided a great opportunity to evaluate this degradation phenomenon. After 21 days, 34% of arsenic was released from S. natans VIII, 33% from S. natans I, and 53% from S. fluitans III. These findings suggest that arsenic release begins after several days of degradation. The levels observed indicate a similar behavior for both S. natans morphotypes. Furthermore, the high liberation of arsenic observed in S. fluitans III and its predominance in holopelagic Sargassum rafts are disturbing.

#### Evaluation of sequential filtration and centrifugation to capture environmental DNA and survey microbial eukaryotic communities in aquatic environments

Atteia, A.<sup>1#</sup>, Bec, B.<sup>2</sup>, Gianaroli, C.<sup>1</sup>, Serais, O.<sup>1</sup>, Quétel, I.<sup>1</sup>, Lagarde, F.<sup>1</sup> & Gobet, A.<sup>1#</sup>\*

<sup>1</sup>MARBEC, Univ Montpellier, CNRS, Ifremer, IRD, Sète, France

<sup>2</sup>MARBEC, Univ Montpellier, CNRS, Ifremer, IRD, Montpellier, France

#These authors equally contributed to the study, ariane.atteia@cnrs.fr, angelique.gobet@ifremer.fr

Sequential membrane filtration of water samples is commonly used to monitor the diversity of aquatic microbial eukaryotes. This capture method is efficient to focus on specific taxonomic groups within a size-fraction but it is time-consuming. Centrifugation is often used to collect microorganisms from pure culture and could be seen as an alternative to capture microbial eukaryotic communities from environmental samples. Here we compared the two capture methods to assess diversity and ecological patterns of eukaryotic communities in the Thau lagoon, France. Water samples were taken twice a month over a full year and sequential filtration targeting the picoplankton (0.2-3 µm) and larger organisms (>3 µm) was used in parallel to centrifugation. An environmental DNA approach targeting the V4 region of the 18S rRNA gene was used to describe the microbial eukaryotic community. The most abundant divisions in the filtration fractions and the centrifugation pellet were Dinoflagellata, Metazoa, Ochrophyta, Cryptophyta. Chlorophyta were dominant in the centrifugation pellet and the picoplankton fraction but not in the larger fraction. Diversity indices and structuring patterns of the community in the two fractions and centrifugation were comparable. Twenty amplicon sequence variants were differentially abundant between the two size-fractions and centrifugation, and their temporal patterns of abundance in the two fractions combined were similar to those obtained with centrifugation. Overall, centrifugation led to similar ecological conclusions as the two filtrated fractions combined, thus making it an attractive time-efficient alternative to sequential filtration.

### A metabarcode based (species) inventory of the northern Adriatic phytoplankton

Grižančić, L.\*, Baričević A., Pfannkuchen, D.M., Smodlaka Tanković, M., Kogovšek, T., Vlašiček, I., Knjaz, M., Podolšak, I. & Pfannkuchen, M.

*Ruder Boskovic Institute, Centre for Marine Research, Rovinj-Rovigno, Croatia* \*lgrizanc@irb.hr

The northern Adriatic is characterized as one of the most productive marine areas in the Mediterranean, which is due to the high levels of nutrients introduced by river discharges, mainly the Italian Po River. The northern Adriatic phytoplankton biodiversity and abundance as a baseline in environmental monitoring is well-studied, through many scientific and longterm reports. These datasets were based on phytoplankton morphological traits traditionally obtained with light microscopy.

Here, metabarcoding methods were used to re-examine taxonomic richness and provide updated knowledge of phytoplankton diversity in the eastern northern Adriatic to complement the standardized light microscopy method, targeting around 430 bp of the V4 hypervariable region of the small subunit ribosomal RNA. Monthly two-year net sampling targeted 7 phytoplankton groups including

Bacillariophyceae, Chrysophyceae, Dinophyceae, Cryptophyceae, Prasinophyceae, Chlorophyceae and Haptophyta.

The most diverse group was dinoflagellates, consisting of 34 genera (48.3%), followed by diatoms with 23 (35.4%) and coccolithophorids with 3 genera (4.0%) found. In terms of genetic diversity, a great majority of sequences with one nucleotide tolerance (ASVs) assigned to species or genus level were dinoflagellates (83.8%), 13.7% diatoms and 1.6% Chlorophyta. The most taxa assigned at the species level belonged to dinoflagellates (42 species or 48.8% of total species) and diatoms (36 species, 41.7%). Chlorophyta and Haptophyta were represented with three species each (3.5%) and Cryptophyta with two species. Metabarcoding revealed a number of previously not listed species for the study area, acting as an important approach in phytoplankton biodiversity assessments.

## The SeaStrains Network – developing a European concept for safeguarding seaweed genetic material to promote food security, biosecurity, and conservation of biodiversity

Hofmann, L.C.<sup>1,2</sup>, Bartsch, I.<sup>1\*</sup> & Brakel, J.<sup>3</sup>

<sup>1</sup>Alfred Wegener Institute Helmholtz Center for Polar and Marine Research, Germany <sup>2</sup>Bremerhaven University of Applied Sciences, Germany <sup>3</sup>Helmholtz Institute for Functional Marine Biodiversity at the University of Oldenburg, Germany \*inka.bartsch@awi.de

One of the main limitations currently preventing the rapid scale-up of seaweed production in Europe is the availability and maintenance of established seaweed strains and cultivars in biobanks. Biobanking of genetic material is a well-established concept for establishing, maintaining and protecting crop lines for land-based agriculture and conserving the gene-pool of wild populations. They preserve genetic diversity, ensure food security, and help preserve historical and cultural value. Nevertheless, seaweeds represent a minute percentage of culture collections, and existing seaweed collections are small, disjoined, often inaccessible, and lack standardization and long-term sustainability. Therefore, we have established the SeaStrains Network, whose mission is to transition from decentralized, poorly documented seaweed stock cultures to a well-documented and easily accessible network of genetically and phenotypically characterized seaweed strains.

The first step to achieving this mission is creating a database of existing seaweed strains in European culture collections. Requests for sharing data using a universal template will be distributed widely to all European seaweed networks. Basic information such as scientific name, Aphia ID, GPS coordinates of the collection site, collection date, collector, associated publications, genetic sequences, and current location of the strain will be consolidated into the database. This openly accessible database will reveal taxa and regions that are strongly or poorly represented, as well as the currently preserved intra- and inter-specific biodiversity. This initiative will also contribute to the planned outcomes of the EU4Algae initiatve and will be an important step towards building bridges between science, policy and inudstry within the seaweed cultivation sector.

### Seasonal dynamics of the protist community in the Oslofjord and Skagerrak in a changing ocean

Kline, S.<sup>1\*</sup>, Eikrem, W.<sup>1,2</sup>, Saubrekka, K.<sup>1</sup>, Egge, E.<sup>2</sup>, Eiler, A.<sup>1</sup>, Andersen, T.<sup>1</sup> & Edvardsen, B.<sup>1</sup>

<sup>1</sup>Department of Biosciences, University of Oslo, P.O. Box 1066 Blindern, Oslo, 0316 Norway <sup>2</sup>Norwegian Institute for Water Research (NIVA), Gaustadalleen 21, 0349 Oslo, Norway \*simonhk@ibv.uio.no

Single-celled protists constitute the majority of the diversity found within the Eukaryota domain of life and contain the oldest form of eukaryote life. As aquatic protists and prokaryotes compose approximately 90% of the biomass in the oceans, any changes within the protist community are likely to have reverberating effects throughout the ecosystem, which in turn may affect the overall resilience of marine ecosystems to changing conditions. In this project we will explore the seasonal diversity and dynamics of the protist community found in the inner and outer Oslofjord by metabarcoding using the latest high-throughput technology (Illumina sequencing and possibly PacBio). We will analyze samples collect monthly during 2023 and re-sequence plankton samples collected during previous research projects since 2009 with present sequencing technology. The current protist community composition in the Oslofjord and Skagerrak will be compared with that from almost 15 years ago.

Globally, coastal marine systems are experiencing increased freshwater discharge from land and a darkening of the water column along with changes in nutrient concentration. This coastal darkening has been a possible explanation of community shifts in similar water systems and in this study, we will therefore measure CDOM (colored dissolved organic matter) and use bioinformatics (such as non-metric multidimensional scaling and ordination analyses) to examine whether browning or any other physico-chemical conditions can explain observed change in the protist community.

#### Resolving cryptic species complexes in marine protists: haplotype networks meet global DNA metabarcoding datasets

De Luca, D.<sup>1</sup>, Piredda, R.<sup>2</sup>, Sarno, D.<sup>1</sup> & Kooistra, W.H.C.F.<sup>1\*</sup>

<sup>1</sup>Stazione Zoologica Anton Dohrn, Italy <sup>2</sup>University of Bari, Italy \*kooistra@szn.it

Marine protists have traditionally been assumed to be lowly diverse and cosmopolitan. Yet, recent studies have shown that many protist species actually consist of cryptic complexes of species whose members are often restricted to biogeographic regions. Detection of cryptic species is usually hampered by sampling coverage and application of methods (e.g. phylogenetic trees) that are not well suited to identify relatively recent divergence and ongoing gene flow. These issues can be overcome by inferring phylogenetic haplotype networks from global metabarcoding datasets. As study case we focus on *Chaetoceros curvisetus* (Bacillariophyta), a globally distributed planktonic species complex within the centric diatoms. Using two complementary metabarcoding datasets (Ocean Sampling Day and Tara Oceans), we resolve the cryptic complex in terms of number of inferred species, and detect new hypothetical species in both datasets. Gene flow is absent between most of the species, but no clear barcoding gap exists either. Some species appear to have restricted distribution patterns whereas others are widely distributed. Closely related taxa occupy contrasting biogeographic regions, suggesting that geographic and ecological differentiation drive speciation. In conclusion, we show the potential of the analysis of metabarcoding data with evolutionary approaches for systematic and phylogeographic studies of marine protists.

#### Diatoms as epiphytes on crustose red algae

Leira, M.<sup>1\*</sup>, Le Gall, L.<sup>2</sup>, Deter, J.<sup>3</sup>, Ballesta, L.<sup>3</sup>, Bárbara, I.<sup>1</sup> & Peña, V.<sup>1</sup>

<sup>1</sup>BioCost Research Group, Department of Biology, University of A Coruña, Spain <sup>2</sup>Institut de Systématique, Évolution, Biodiversité (ISYEB), Muséum National d'Histoire Naturelle, CNRS, Sorbonne Université, EPHE, Université des Antilles, Paris, France <sup>3</sup>Andromède océanologie, Mauguio, France \*m.leira@udc.es

In marine ecosystems, crustose red algae (also known as corallines) have a global distribution and a wide bathymetric range, able to live in mesophotic ecosystems with little light penetration. Their most characteristic feature is the calcification of their cell walls which is interpreted as an evolutionary novelty that favoured their radiation mainly by conferring defense against grazing. For all these characteristics, they are considered ecosystem engineers that promote a high biodiversity of associated fauna and flora. Less known, however, is the diversity of microorganisms associated, and in particular, the diatom community able to grow as epiphytes on these crustose red algae. Planktonic diatoms have been long collected from marine environments, but almost no data exist for epiphytic communities despite their high ecological significance and enormous scientific potential.

Here, we present a preliminary study on the composition of diatom community associated with crustose red algae collected in various localities in the Atlantic and Mediterranean, from shallow subtidal to deeper areas (>90m depth). We performed this study to gather baseline information on the species composition of diatom communities and determine if some environmental variables might have some influence on their distribution patterns. Epiphytic diatoms were studied by means of scanning electron microscopy. In terms of species composition, there were major differences between the diatom communities on the different crustose rhodophyte hosts. Motile (mainly smal-celled) and adnate (*Cocconeis* spp.) diatoms dominated the community. We test whether the bio-physicochemical characteristics of each sampling site affected the epiphytic diatom communities more than the substrate type provided by the macroalgal host or the sampling depth.

#### Microphytobenthos communities living on *Laminaria* forests by DNA metabarcoding

Leira, M.1\*, López-Rodríguez, M.C.<sup>2</sup>, Barrientos, S.<sup>1</sup> & Barreiro, R.<sup>1</sup>

<sup>1</sup>BioCost Research Group, Department of Biology, University of A Coruña, Spain <sup>2</sup>BioAplic Group, Department of Botany, University of Santiago de Compostela, Spain \*m.leira@udc.es

*Laminaria* forests are highly relevant for coastal ecosystems as they provide a diverse range of ecological services. In addition to their ecological importance, they also have economic and cultural significance. Unfortunately, *Laminaria* forests are under threat from a variety of human activities. Their loss could have significant impacts on coastal ecosystems and the communities that depend on them.

Microphytobenthos (MPB) communities living on *Laminaria* can provide valuable information about the health and condition of kelp forests. Changes in their composition and abundance can indicate changes in environmental conditions such as nutrient availability, light penetration, and sedimentation. Therefore, monitoring these MBP communities can provide important insights into the health and condition of kelp forests.

The arrival of HTS technologies potentially makes studying these aspects more feasible. However, DNA metabarcoding of environmental samples has been rarely tested in coastal ecosystems to study MPB and

the few studies to date indicate a fair agreement with those using morphological approaches. We compare the efficiency of combining LM-based identifications and DNA metabarcoding, based on rbcL and ITS markers, for examining MPB communities collected from *Laminaria* specimens in Atlantic shallow coastal environments. We show that this MPB community is highly diverse and that DNA metabarcoding effectively reflects this diversity at the genus rather than the species level because DNA reference libraries are incomplete. Nevertheless, DNA metabarcoding offers a strong support helping to identify and classify species which are difficult to distinguish based on their physical characteristics, and resolving taxonomic disputes and identifying new species.

#### PHYTOBS-Network: French National Service of Observation program for Phytoplankton in coastal waters

Lemoine, M.<sup>1\*</sup>, Claquin, P.<sup>2</sup> & all contributors from marine stations

<sup>1</sup>*IFREMER Nantes, France* <sup>2</sup>*Université de Caen Normandie BOREA – CREC, France* \*maud.lemoine@ifremer.fr

PHYTOBS-Network is a French national microphytoplankton monitoring network belonging to Coastal Ocean and Nearshore Observation French Research Infrastructure ("ILICO"). PHYTOBS-Network combines resources and skills from the French institutes Ifremer, CNRS and universities.

The objectives of this network are to analyze the responses of phytoplankton communities to environmental changes, assess the quality of the coastal waters through indicators, define ecological niches, and detect phenological variations. PHYTOBS also offers a synergy within the scientific community through networking and allows the dissemination and consolidation of skills.

PHYTOBS-Network relies on 25 sites distributed along the French coast. The monitoring started in 1987 on some sites. It consists in studying microphytoplankton assemblages and associated hydrological parameters.

The PHYTOBS-network provides the scientific community and stakeholders with long term series, in order to improve knowledge regarding biomass, abundance and composition of marine microphytoplankton in coastal waters in their hydrological context. The PHYTOBS data set is very original regarding the two types of taxonomic nomenclatures it proposes. A classical one, with raw data, where the highest taxonomic level reached by each analyst depends on each one's abilities. In order to standardize taxon groupings, the PHYTOBS-

Network also provides an "accredited taxon" dataset corresponding to the best level of identification the whole French analysts community is able to guarantee.

Sharing standardized sampling and analyzing methods, PHYTOBS-Network data are an answer to the need of long-term observation of microphytoplankton communities along the French coasts. It provides researchers and end-users with taxonomic information from raw taxon list to an accredited taxon level.

### Genus *Asparagopsis* (Bonnemaisoniales, Rhodophyta) in the Croatian part of the Adriatic Sea

Lučić, P.\*, Žuljević, A., Cvjetan, S., Nejašmić, J., Despalatović, M. & Cvitković, I.

In the Adriatic Sea two non-indigenous species of the genus Asparagopsis Montagne (Bonnemaisoniales) are present, A. armata Harvey (recorded in the late 1970s) and A. taxiformis (Delile) Trevisan (recorded in 2000). These tropical-subtropical red algae have a heteromorphic life history with an erect gametophyte and a filamentous tetrasporophyte phase, which has even been considered a separate species named Falkenbergia. Both tetrasporophytes are morphologically similar and cannot be easily distinguished. Until now, all Falkenbergia specimens collected in the Adriatic Sea were assigned to A. armata exclusively based on morphology. The aim of this study was to investigate the distribution of tetrasporophytes of both species within the Croatian part of the Adriatic Sea using DNA barcoding based on chloroplast rbcL and mitochondrial cox2-3 spacer. The results revealed that all the collected Falkenbergia belong to the tetrasporophyte of A. taxiformis, except from a single restricted area where A. armata was detected. Interestingly, this is also the only site within the Adriatic Sea where the gametophyte of A. armata was detected for the first and last time in the early 1990s. Our results indicate that most of the recent Falkenbergia records of A. armata may have been misidentified. Asparagopsis taxiformis is more extensively widespread in the Adriatic Sea than we previously assumed based only on gametophyte phase detection while A. armata can be considered as rare. It is quite possible that A. armata declined after initial spreading and was replaced over time by the tetrasporophytes of A. taxiformis.

#### Species diversity and distribution of the genus *Jania*, at global scale with a European focus

Maridakis, C., Le Gall, L. & Rousseau, F.

Équipe Exploration, Espèces et Évolution, Institut de Systématique, Évolution, Biodiversité, UMR 7205 ISYEB CNRS, MNHN, UPMC, EPHE, Muséum national d'Histoire naturelle (MNHN) clio.maridakis@mnhn.f; line.le-gall@mnhn.fr; florence.rousseau@mnhn.fr

The coralline algae are characterized by the presence of calcite in their cell walls, a worldwide distribution on most of costal habitats and have been reported from the intertidal to depth of 268 meters. Coralline algae are among the most species diverse group of red algae and contribute important ecological roles in the marine ecosystem. The phenotypic plasticity and morphologic convergences of the coralline algae render molecular systematic mandatory to clarify species boundaries and diversity. In the present study we focused on Jania because among coralline algae Jania is the fourth most diverse genus after Lithophyllum, Lithothamnion and Amphiroa, and is relatively easy to identify at a generic rank whereas the others are not. Historically the tribe Janieae included the genera Jania, Cheilosporum and Haliptilon, however based on molecular and morphological characters, Cheilosporum and Haliptilon were subsumed to Jania. Therefore the 55 accepted species of the tribe Janieae currently all belong to the genus Jania. In the present study we aimed at evaluating species diversity within the genus Jania at a global scale based on the collection of the National Herbarium of Muséum national d'Histoire Naturelle (PC) which consist of 300 specimens of Jania. In addition, we included sequences available from GenBank and BOLD to delineate species and infer their phylogenetic relationships based on the plastid gene psbA and the mitochondrial gene COI. We will present the results of species diversity and distribution of Jania with a focus on European Atlantic and Mediterranean seas.

### Freshwater ecosystem bioengineer on a small and large scale (Cladophora, Chlorophyta)

Messyasz, B.

Department of Hydrobiology, Faculty of Biology, Adam Mickiewicz University in Poznan, Uniwersytetu Poznanskiego 6, 61-614 Poznan, Poland messyasz@amu.edu.pl

Ecosystem engineers can create or modify habitats by affecting environmental parameters, such as light, temperature, pH, oxygen, availability of substrate. *Cladophora glomerata* (L.) Kütz. is a widespread filamentous alga, occurring in a broad range of freshwater habitats and is an example of structural ecosystem engineers (autogenic) that provides a multitude of ecological niches for associated organisms along small-scale and large-scale environmental gradients. The mass development of filamentous Cladophora is regulated by its ecological preferences in relation to environmental factors and to variations of these in time and space.

At the same time, such an intense appearance of macroalgae usually contributes to a high degree to physical, chemical and biological disturbances in the habitat for microalgae (phytoplankton, periphyton, microphytobenthos). In this study, attention was given to changes of species composition, abundance and biomass of different ecological microalgae groups as a result of mass development of macroalgae. Two aquatic ecosystems were compared: the shallow Lake Oporzynskie and the small lowland river Nielba, both with seasonally occurring large biomass of Cladophora but different hydrological characteristics. Our results indicate that the benthic microalgal communities in both aquatic ecosystems are more diverse and stable than the phytoplankton communities. The diversity and seasonal dynamics are presented in this work.

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#### Seagrass vs. green macroalgae discrimination: from a hyperspectral library to Drone mounted multispectral camera

Oiry, S.<sup>1\*</sup>, Rowe Davies, B.F.<sup>1</sup>, Sousa, A.<sup>2</sup>, Gernez, P.<sup>1</sup>, Rosa, P.<sup>1</sup> & Barillé, L.<sup>1</sup>

<sup>1</sup>Nantes Université, Institut des Substances et Organismes de la Mer, ISOMer, UR2160, Nantes F-44000, France

<sup>2</sup>CESAM – Centre for Environmental and Marine Studies, Department of Biology, University of Aveiro, Campus Universitário de Santiago, Aveiro 3810-193, Portugal \*oirysimon@gmail.com

This study aims to apply drone technology for high-resolution mapping of intertidal seagrass meadows. Most of the green macrophytes growing on the intertidal area share the same pigmentary composition, making the discrimination between them challenging using remote sensing. The spectral signature of these vegetation types (e.g. *Ulva* sp. and *Zostera noltei*) are very similar within visible wavelengths, but slight differences can be observed in the nearinfrared. High spectral resolution tools, like field spectroradiometers, used to collect hyperspectral signatures, efficiently distinguish seagrass from green macroalgae. However, the spatial resolution of satellite mounted hyperspectral sensors is too coarse (~30 m) to accurately map small patches of intertidal vegetation. This is especially true in complex or heterogeneous areas, while hyperspectral drone-mounted sensors are resource and time intensive.

To overcome these limitations, we investigated the potential of multispectral data acquired by drones to map intertidal vegetation at a very high spatial resolution. Our results showed that degrading hyperspectral signatures to the spectral resolution of a MicaSense Dual Sensor camera (10 spectral bands ranging from 444nm to 840nm) allows the discrimination of macrophytes. Our study demonstrates that combining drones, multispectral data, and neural networks is a promising approach for mapping intertidal vegetation, notably discriminating seagrass meadows and macroalgae. Some confusion arises

between seagrass and xanthophytes (*Vaucheria* sp.). This method provides a cost-effective and efficient way to map the macrophyte biodiversity at the class taxonomic resolution, and it can potentially inform management strategies of these ecosystems.

### Marginal hotspots of genetic diversity of red gold *Gelidium corneum* along its biogeographical range

Potin, A.\*, Neiva, J. & Serrão, E.A.

Center of Marine Sciences, CCMAR-CIMAR, University of Algarve, Faro, Portugal \*anjelina.potin@imbrsea.eu

Many species present unique lineages and gene pools at their low-latitude range limits, at habitats that have persisted during past climates. Climate change is predicted to cause a poleward shift for most species, threatening such low latitude relict diversity. We investigated the genetic structure of *Gelidium corneum* (Rhodophyta), the most valuable species for the laboratory agar industry, commonly called red gold due to its high prices in the blueeconomy. This species is distributed from Mauritania to Great Britain. Overexploitation and climate change are likely reasons for the drastic declines in the past and are predicted to continue so. This may lead to population collapses at the southern range, potentially impacting the genetic diversity of *Gelidium corneum*.

Thus, we aimed to assess the genetic structure of *G. corneum* along its entire biogeographical range, using microsatellites.

Results revealed two main genetic clusters with little evidence of admixture, from northern Spain to Brittany, and from Portugal to Mauritania. These showed similar levels of allelic and genotypic diversity, and intra-regional population divergence, although more private alleles were found in the southern region. Most populations showed a very low number of homozygotes which suggests that populations are dominated by tetrasporophytes. Most populations however exhibited significant heterozygote deficiencies which could be related to the low dispersal range and local inbreeding.

Our results highlight the importance of considering intraspecific genetic diversity in the selection of populations for the industrial production of *G. corneum*. This is especially relevant for isomorphic species and in the context of climate-driven range shifts.

### Experimental demonstration of recovery capacity of *Gelidium corneum* populations based on lab-induced reattached individuals

Sánchez-Astráin, B.\*, Sainz-Villegas, S., Guinda, X., de la Hoz, C.F., Puente, A. & Juanes, J.A.

IHCantabria - Instituto de Hidráulica Ambiental de la Universidad de Cantabria, Spain \*sanastrainb@unican.es

The macrophyte *Gelidium corneum* plays an important role as an ecosystem engineering in temperate rocky shores. Its wide-scale harvesting is attributed to the high-quality agar production that it yields. However, in the last decades, this canopy-forming alga is facing local declines in the Cantabrian coast most likely due to increasing temperatures and irradiance.

We carried out an *in-situ* experiment to study the growth process of new individuals from reattached apical fragments of *G. corneum*. Characterization of *G. corneum*'s vegetative propagation has been analyzed in a previous study (C3N-pro project), on whose results this investigation is based. The

experiment was settled at two depths within the range of distribution of this species (5 and 12 meters) and two coastal sites characterized by differences in their temperatures (east and west coast of Cantabria). Each area and depth contained 2 stainless steel structures with 24 experimental substrates. Growth capacity was estimated at both sites, at monthly intervals approximately, from September to December 2022 by the sequential removal of 8 experimental substrates per each depth and location at each sampling period. Once in laboratory, number of shoots and length were quantified.

Our results show that *G. corneum* apical fragments develop a large number of new individuals (8,658 in total), although an unexpected variable had a significative influence, herbivory. This leads us to think in prototypes applied in ecological restauration of disturbed fields of this species. Further research should be done on parametrizing this data to include them in hybrid species distribution models.

### The genus *Cystoseira* s.l. in Israel (the Levantine basin of the Mediterranean Sea)

#### Sadogurska, S.S.<sup>1</sup>\* & Israel, Á.<sup>2</sup>

<sup>1</sup>Department of Phycology, Lichenology and Bryology, M.H. Kholodny Institute of Botany NASU, Ukraine <sup>2</sup>Israel Oceanographic & Limnological Research (IOLR), the National Institute of Oceanography, Israel \*s.sadogurska@gmail.com

In this work, we present the results of the comprehensive taxonomic revision of *Cystoseira* s.l. (Phaeophyceae) taxa found on the Mediterranean coast of Israel (Levant Basin), and clarify their taxonomic diversity and distribution. Using comparative morphological and molecular phylogenetic methods, we analyzed fresh seaweed samples collected in 2019 from nine sites along the Israeli shores in the intertidal (including vermitid reefs) and subtidal (2-12 m), as well as dried samples from the Israel National Herbarium of the Hebrew University of Jerusalem (HUJ) and the Seaweed Herbarium at the Israel Oceanographic and Limnological Research Institute (IOLR).

The revised fresh samples showed the presence of two genera, namely, *Cystoseira* s.s. and *Gongolaria*. Three taxa belong to the genus *Cystoseira* s.s.: *Cystoseira compressa* (Esper) Gerloff & Nizamuddin, widely spread along Israeli shores, and two rare taxa: *Cystoseira foeniculacea* (Linnaeus) Greville, the first well-documented finding in the last 25 years, and *Cystoseira pustulata* (Ercegović) Neiva & Serrão, described for the Mediterranean Israeli shores for the first time.

Morphological and molecular-phylogenetic approaches allowed to clarify the species diagnosis for the endemic Levantine species *Gongolaria rayssiae* (Ramon) Molinari & Guiry. The morphological variability, most pronounced between specimens growing in the subtidal and tidal zones, was described. The analysis of herbarium specimens showed that in the early XX century, *Gongolaria barbata* (Stackhouse) Kuntze and *Ericaria barbatula* (Kützing) Molinari & Guiry species also occurred in Israel, but currently they are likely extinct.

Altogether, this current study confirms the presence of six species from the genera *Cystoseira* s.s., *Ericaria* and *Gongolaria* of which three were identified as new species for Israel (from which two most probably have already disappeared).

### First data on a green coccoid microalga isolated from Lido of Spina (Comacchio, Ferrara, Italy)

Sciuto, K.<sup>1</sup>\*, Sfriso, A.A.<sup>1</sup>, Wolf, M.A.<sup>2</sup>, Sfriso, A.<sup>2</sup>, Moschin, E.<sup>3</sup>, Munari, C.<sup>1</sup>, Mistri, M.<sup>1</sup> & Moro, I.<sup>3</sup>

<sup>1</sup>Department of Chemical, Pharmaceutical and Agricultural Sciences, University of Ferrara, Italy

<sup>2</sup>Department of Environmental Sciences, Informatics and Statistics, Ca' Foscari University of Venice, Italy <sup>3</sup>Department of Biology, University of Padova, Italy \*katia.sciuto@unife.it

Microalgae are a wide group of photoxygenic microorganisms belonging to different taxa, able to colonize several habitats and showing different adaptations to environmental conditions. Many of the compounds produced by microalgae can be employed in human fields, such as agriculture, nutraceutics and cosmeceutics, with generally null or scarce impact on the environment and/or resource consumption. In this context, the research project "Biotechnological potentials of microalgae for environmental sustainability" has been recently founded by the European Social Fund (ESF) - Italian National Operational Programme (NOP) on Research and Innovation 2014-2020. The project aim is to characterize several microalgal strains in order to understand their possible use in human fields, considering both strains already available in public/private collections, but only partially characterized, and new strains isolated from natural environments, with a particular attention to marine and transitional water habitats.

First samplings were carried out in the coastal area of Lido of Spina (Comacchio, Ferrara, Italy), both along the coast and from more internal water basins. Once in the laboratory, water samples were processed and used for microalgal isolation. Among the isolates, a green coccoid microalga, temporarily named strain KS2 and sampled from one of the internal water basins, was found. The microalga was subjected to a polyphasic approach, including molecular (based on the 18S rRNA gene and the 18S-28S ITS rRNA region), morphological (LM and SEM), ultrastructural (TEM) and biochemical (e.g., pigments, proteins, polysaccharides) surveys. Here we report the first results of this characterization.

#### Mapping and DNA barcoding the brown algal diversity within the orders Ectocarpales, Ralfsiales and Sphacelariales in Norway

Sjøtun, K.<sup>1\*</sup>, Fredriksen, S.<sup>2</sup>, Gabrielsen, T.M.<sup>3</sup>, Husa, V.<sup>4</sup>, Goecke, F.<sup>5</sup>, Le Gall, L.<sup>6</sup>, Rousseau, F.<sup>6</sup>, Bringloe, T.T.<sup>7</sup> & Parente, M.I.<sup>8</sup>

<sup>1</sup>University of Bergen, Norway

<sup>2</sup>University of Oslo, Norway

<sup>3</sup>University of Agder, Norway

<sup>4</sup>Institute of Marine Research, Norway

<sup>5</sup>Norwegian University of Life Sciences

<sup>6</sup>Muséum national d'Histoire naturelle, France

<sup>7</sup>Department of Fisheries and Oceans Canada, Canada

<sup>8</sup>CIBIO, InBIO Laboratório Associado, BIOPOLIS; UNESCO Chair – Land Within Sea and Faculdade de Ciências e Tecnologias, Universidade dos Açores, Portugal

Among brown algae, the order Ectocarpales is the most species rich. It comprises around 108 recorded marine species in mainland Norway, however the knowledge about many of these species is rather limited. In addition, the knowledge about the tuft- and crust-forming taxa of the orders Ralfsiales and Sphacelariales is also poor. A project funded by the Norwegian Biodiversity Information Centre on mapping and DNA barcoding of brown algae has the goal to collect georeferenced samples of morphological species within these taxonomic groups in Norway and to characterize and DNA barcode these.

The project started in 2021 and will run until 2024, including several national and international partners. During the project's first year, field sampling was initiated in South Norway, which will continue in 2023 and be extended to North Norway. Samples of several microscopic Ectocarpales have been

cultured. The collected samples are now being DNA barcoded (COI-5P), and preliminary results show an increased species diversity in the genera *Scytosiphon*, *Petalonia*, *Punctaria* and *Pseudoralfsia* compared to previous records in Norway. In addition to standard barcoding, a trial with metabarcoding is being carried out on collected material from one site.

The web site http://seaweeds.uib.no is used as the main platform for the dissemination of information related to the project.

### From species to community: long-term dynamics of phytoplankton biodiversity across environmental gradients

Skejić, S.<sup>1</sup>\*, Matić, F.<sup>2</sup>, Bakrač, A.<sup>1</sup>, Straka, M.<sup>1</sup>, Bužančić, M.<sup>1</sup>, Arapov, J.<sup>1</sup> & Ninčević Gladan, Ž.<sup>1</sup>

<sup>1</sup>Institute of Oceanography and Fisheries, Croatia <sup>2</sup>University Department of Marine Studies, Croatia \*sanda@izor.hr

Phytoplankton can serve as a good indicator of the state of the marine environment. By analyzing longterm phytoplankton data over 14 years, we describe the interannual, seasonal and vertical variability of 2291 samples along transect of four stations in the central Adriatic Sea, trying to identify responses caused by anthropogenic or climate changes.

We used a neural gas algorithm to classify the heterogeneous phytoplankton community in terms of the contribution of 4 phyla (Bacillariophyta, Miozoa, Haptophyta, and Phytoflagellates) to the overall community and distinguish five best-matched units BMUs. BMU5 describes the most common pattern in our samples (29.5%) with predominant phytoflagellates found mainly in open waters, while BMU3 and BMU4 with predominant Bacillariophyta are more commonly found in coastal stations. These clusters accounted for 18% and 22% of the proportion of samples analyzed, respectively. BMU1 accounts for only a small fraction of the samples with increased Haptophyta (particularly in 2017 and 2018 at open stations).

The Bacillariophyta show no increasing trend in abundance over the years (2007-2020) and are more common in the coastal stations. In contrast to the Bacillariophyta, the Haptophytes show an increasing tendency and occur more frequently at the open stations. A large number of taxa emerge over the course of the surveys (462 taxa), with a clear upward trend in the number of taxa for each station and consequently an increasing Margalef diversity index. This is particularly evident from 2014 onwards. The Shannon and Pielou indices are more consistent, although lower at the open stations

### Diversity and seasonality of coccolithophores in Slovenian coastal waters (Adriatic Sea)

Slavinec, P.<sup>1,2</sup>\*, Fortič, A.<sup>1</sup>, Francé, J.<sup>1</sup> & Mozetič, P.<sup>1</sup>

<sup>1</sup>National Institute of Biology, Marine Biology Station Piran, Slovenia <sup>2</sup>University of Ljubljana, Biotechnical Faculty, Slovenia \*petra.slavinec@nib.si

Coccolithophores are a common marine phytoplankton characterized by calcium carbonate plates (coccoliths) and play an important role in the global carbon cycle.

In the Slovenian part of the Gulf of Trieste (northern Adriatic Sea), 16 coccolithophore taxa were identified prior to this study during routine long-term monitoring based on morphological identification

by light microscopy, but the seasonal and spatial (along the water column) distribution of life cycle stages, haploid holococcolith vs. diploid hetereocccolith stage, was not considered.

This is the first in-depth study of coccolithophores in the Slovenian Sea. Seawater samples for morphological identification and metabarcoding have been collected every two weeks since October 2022 at the LTER sampling site, where environmental conditions are always monitored, at two depths: 0 m and 15 m. Coccolithophores are identified using an inverted light microscope and a scanning electron microscope. Morphological identification will be compared and complemented with metabarcoding analysis.

Preliminary results show that the diversity of coccolithophores in the Slovenian Sea is greater than previously known (21 species to date) and is expected to increase further once the seasonal cycle is complete. Different microscopic techniques allow us to distinguish between holococcolithophores and heterococcolithophores and even specimens with combined coccosphres, which is evidence of the ability of coccolithophores to undergo life cycle transitions.

#### Biomass increase and maturation of North Sea kelp forest despite ocean warming

Steinberg, R.<sup>2</sup>\*, Schubert, P.<sup>2</sup>, Karez, R.<sup>3</sup>, Wiltshire, K.<sup>4</sup>, Bischof, K.<sup>2</sup> & Bartsch, I.<sup>1</sup>

<sup>1</sup>Alfred-Wegener Institute, Helmholtz Centre for Polar and Marine Research, Am Handelshafen 12, 27570 Bremerhaven, Germany

<sup>2</sup>University of Bremen, Marine Botany, Leobener Str. NW2, 28359 Bremen, Germany

<sup>3</sup>GEOMAR, Helmholtz Centre for Ocean Research Kiel, Düsternbrooker Weg 20, 24105, Kiel, Germany

<sup>4</sup>State Agency for Environment Schleswig-Holstein, Hamburger Chaussee 25, 24220, Flintbek, Germany \*rsteinbe@uni-bremen.de

Kelp forests are key components along rocky coastal ecosystems significantly contributing to their production and biodiversity. These ecosystems host an even greater variety and higher diversity of algae and animals than almost any other ocean community. Global warming, eutrophication, sedimentation and pollution are a threat to these habitats and are partially responsible for their global decline. Temperature thereby is a key abiotic factor for survival, growth, and reproduction of kelps as well as for their biogeographical distribution and has steadily risen at our North Sea investigation site (Helgoland, Germany) since 1962. Between 1970 and 2005 the Laminaria hyperborea kelp forest at Helgoland had increased its biomass and depth extension, possibly due to reduced eutrophication and clearer waters since the 1970's. We were interested whether this situation continued or was possibly halted or reversed due to steadily increasing mean seawater temperatures. We repeated the study of 2005 by replicate (n=3)destructive sampling (1 m<sup>2</sup> each) along a depth gradient at 2, 4, 6, 8 and 10 m MLWS. We analyzed the overall macroalgal biodiversity, the biomass of kelps and understorey species, the leaf area, density, age, stipe, and blade length of kelps. The mean kelp biomass integrated over all depths increased significantly from  $0.88 \pm 0.84$  kg DW m<sup>-2</sup> to  $1.06 \pm 0.79$  kg DW m<sup>-2</sup> between 2005 and 2018. The increase in kelp biomass was particularly pronounced in deeper areas (6-10 m MLWS). This biomass increase was accompanied by a significantly increased kelp leaf area index (LAI) (2005: 1.9, 2018: 3.3). Kelps also increased their depth extension. In 2005 no kelps were present at 10 m, while in 2018 kelps were recorded at  $\geq 10$  m MLWS. The kelp age also doubled on average significantly from 2 years to 4 years. Additionally, the understorey seaweed biomass integrated over all depths significantly increased from 0.04 kg DW m<sup>-2</sup> to 0.11 kg DW m<sup>-2</sup> between 2005 and 2018. Furthermore, the temperature and Secchi data from the Helgoland Road time series were evaluated. The temperature data revealed that the winters in the years before 2018 were warmer than the average for the entire period from 2001 to 2018. This could have enhanced growth. Despite the global trend, the kelp forest of Helgoland seems to have benefitted from rising temperatures. Especially the warmer winters potentially have supported the kelp growth and distribution around Helgoland

#### Habitat suitability of rhodolith seabeds in an oceanic archipelago: regional-scale physical and physiological drivers

Triay-Portella, R.<sup>1,2\*</sup>, Hernández, S.<sup>3</sup>, Blanco González, M.<sup>3</sup> Bosch, N.<sup>1</sup>, Tuya, F.<sup>1</sup>, Haroun, R.<sup>1</sup> & Martínez, B.<sup>3</sup>

<sup>1</sup>Grupo de Biodiversidad y Conservación (BIOCON), Instituto Universitario de Investigación en Acuicultura Sostenible y Ecosistemas Marinos (IU-ECOAQUA), Universidad de Las Palmas de Gran Canaria, Spain <sup>2</sup>MARE – Marine and Environmental Sciences Centre / ARNET - Aquatic Research Network, Agência Regional para o Desenvolvimento da Investigação, Tecnologia e Inovação (ARDITI), Portugal <sup>3</sup>Departamento de Biología y Geología, Física y Química Inorgánica, Universidad Rey Juan Carlos, Móstoles, Spain

raul.triay@ulpgc.es

Rhodolith seabeds are key habitats worldwide and play relevant role to climate regulation through their role in sequestering and storing "blue" carbon. Understanding the factors that rule the presence of these habitats is essential for predicting their geographical distribution on a regional scale. Here we explore the habitat suitability of rhodolith seabeds by means of correlative Species Distribution Modelling (SDM) at regional scale. Located west of the African coast, at 28° latitude, the Canary Islands comprise seven islands that emerged after successive volcanic events during the last ~ 20 million year. The multidisciplinary POPCORN project combines efforts to obtain all the cartographic and historical information on the presence of rhodoliths seabeds in Canary Islands, as well as the use of scientific diving techniques and underwater ROVS to validate the correlative SDMs at fine resolution. Spatial scale is important for understanding ecological and biogeographical processes and guiding conservation planning. Variable selection at regional scale and the importance of physical factors in the configuration of rhodoliths seabeds will be explored. Leveraging SDMs as sampling tools will further enhance and make regional models more robust. Correlative SDM based on brute force variable selection and tuned MaxEnt algorithm contribute to a robust final model able to project the habitat suitability of rhodolith seabeds in the Macaronesia Ecoregion.

### The PR<sup>2</sup> ecosystem: a set of interconnected databases to map the distribution of micro-algal taxa

Vaulot, D.\*1, del Campo, J.2, Sim, C.W.H.3 & Lopes dos Santos, A.3

<sup>1</sup>CNRS, Sorbonne Université, UMR 7144, ECOMAP, Station Biologique, 29680 Roscoff, France

<sup>2</sup>Institute of Evolutionary Biology (CSIC - Universitat Pompeu Fabra), Barcelona, Spain

<sup>3</sup>Asian School of the Environment, Nanyang Technological University, 50 Nanyang Avenue, Singapore 639798

\*vaulot@gmail.com

Metabarcoding of the 18S rRNA gene has become the method of choice for investigating the composition and dynamics of microbial eukaryotic communities. Marker gene amplification by PCR and annotation of the sequences are at the core of metabarcoding. A decade ago, the PR<sup>2</sup> database

(https://app.pr2-database.org) was developed. It contains reference 18S rRNA sequences and is regularly updated by experts for a range of taxonomic groups. Currently, PR<sup>2</sup> provides the most accurate taxonomic annotation of metabarcodes down to near species level. The PR<sup>2</sup> database is coupled with a primer database (https://app.pr2-primers.org) that allows testing amplification primers against PR<sup>2</sup> reference sequences.

A multitude of environmental metabarcoding datasets are being published. However, processed data, i.e. clustered sequences such as amplicon sequence variants (ASVs), are rarely available in standard format. To fill this gap, we recently developed metaPR<sup>2</sup> (https://app.metapr2.org), a database of processed 18S rRNA metabarcodes that are taxonomically annotated with the PR<sup>2</sup> database. Version 2.0 of MetaPR<sup>2</sup> contains 59 datasets corresponding to more than 6,200 samples and 93,000 ASVs.

These interconnected databases should prove very useful to all researchers working on protist and in particular microalgal diversity across a variety of ecosystems. We will present these resources through examples focusing on algal distributions and chart future developments, in particular linked to long read sequencing and addition of functional traits such as trophic status.

#### SYMPOSIUM 03 "Phylogenetics, Phylogenomics and Evolution"

#### The Phaeoexplorer Genome Database: a Multi-Scale Genomic and Transcriptomic Data Resource for the Brown Algae

Brillet-Guéguen, L.<sup>1,2</sup>\*, Le Bars, A.<sup>3,2</sup>, Dallet, R.<sup>2</sup>, Le Corguillé, G.<sup>2</sup>, Godfroy, O.<sup>1</sup>, Coelho, S.M.<sup>1,4</sup>, Corre, E.<sup>2</sup> & Cock, J.M.<sup>1</sup>

 <sup>1</sup>Sorbonne Université, CNRS, Integrative Biology of Marine Models (LBI2M), Station Biologique de Roscoff (SBR), 29680, Roscoff, France
 <sup>2</sup>CNRS, Sorbonne Université, FR2424, ABiMS, Station Biologique, 29680, Roscoff, France
 <sup>3</sup>CNRS, Institut Français de Bioinformatique, IFB-core, UMS 3601, Évry, France
 <sup>4</sup>Max Planck Institute for Biology, Department of Algal Development and Evolution, Tübingen, Germany
 \*loraine.gueguen@sb-roscoff.fr

The Phaeoexplorer project has generated more than 60 annotated genome assemblies, together with transcriptome data, for a broad range of brown algal species to address key questions about their biology and evolutionary history.

To provide the community with a collaborative hub for accessing, visualizing and analyzing the brown algal genome and transcriptome resources, we have developed a web portal (https://phaeoexplorer.sbroscoff.fr) to house the annotated genome sequences along with a broad range of associated resources, including an integrated environment based on the Galaxy Genome Annotation project dedicated to the visualization of genomic data, with genome browsers (https://doi.org/10.7490/f1000research.1116992.1); information about the sequenced strains; assembly and annotation metrics; data download facilities; BLAST facilities; and a web application designed to explore RNAseq data of the model alga Ectocarpus. We plan to extend the Phaeoexplorer database with additional resources (experimental protocols, genomes of associated bacterial symbionts, comparative genomics). Still partially restricted, in the long term, the objective is for the Phaeoexplorer database to be a user-friendly public access point to brown algal genomes for the entire phycology community, with regular genome releases.

A similar database has been implemented for red algae and another database is planned for fungi. And, within the context of the European Reference Genome Atlas project, in partnership with two other Breton bioinformatics platforms, we are setting up an automated, modular, and FAIR system for the provision, visualization and processing of large-scale genome data: the BEAURIS pipeline (https://gitlab.com/beaur1s/beauris).

#### The Rhodoexplorer Genome Database: a Multi-Scale Genomic and Transcriptomic Data Resource for the Red Algae

Brillet-Guéguen, L.<sup>1,2</sup>\*, Wörtwein, T.<sup>4</sup>, Le Bars, A.<sup>3,2</sup>, Lipinska, A.P.<sup>1,4</sup>, Krueger-Hadfield, S.A.<sup>5</sup>, Godfroy, O.<sup>1</sup>, Dittami, S.<sup>1</sup> & Corre, E.<sup>2</sup>

<sup>1</sup>Sorbonne Université, CNRS, Integrative Biology of Marine Models (LBI2M), Station Biologique de Roscoff (SBR), 29680, Roscoff, France

<sup>2</sup>CNRS, Sorbonne Université, FR2424, ABiMS, Station Biologique, 29680, Roscoff, France

<sup>3</sup>CNRS, Institut Français de Bioinformatique, IFB-core, UMS 3601, Évry, France

<sup>4</sup>Max Planck Institute for Biology, Department of Algal Development and Evolution, Tübingen, Germany

<sup>5</sup>Department of Biology, University of Alabama at Birmingham, 1300 University Blvd, Birmingham, AL, 35294 \*loraine.gueguen@sb-roscoff.fr

Red algae are of interest for a number of aspects, including their life cycles and reproductive biology, as domesticated and cultivated species, as invasive species, and for their characteristic cell walls. The Rhodoexplorer project aims to explore the evolution of biological complexity in the red algae through the establishment of a multi-scale genomic data resource for the red algae, including public data and new sequenced and annotated genomes. The Rhodoexplorer project involves an international consortium including partners at the Max Planck Institute (MPI) in Tübingen (Germany), the Roscoff Biological Station (France), the University of Sao Paulo (Brazil), Universidad Austral de Chile (Chile), the University of Alabama at Birmingham (USA), the University of Charleston (USA), GEOMAR, Kiel (Germany) and the University of Oldenburg (Germany).

The ABiMS platform has developed a new web portal (https://rhodoexplorer.sb-roscoff.fr) to house the annotated genome sequences and associated resources, including genome browsers; information about the sequenced strains; assembly and annotation metrics; data download facilities; and BLAST facilities. In the context of other local genome portal projects, and of the European Reference Genome Atlas project, in partnership with two other Breton bioinformatics platforms, we are setting up an automated, modular, and FAIR system for the provision, visualization and processing of large-scale genome data: the BEAURIS pipeline (https://gitlab.com/beaur1s/beauris).

#### LncPlankton: an extensive collection of IncRNAs from marine plankton

Debit, A.<sup>1\*</sup>, Vincens, P.<sup>2</sup>, Bowler, C.<sup>2</sup> & Cruz de Carvalho, H.<sup>2</sup>

<sup>1</sup>Institut de Biologie de l'ENS (IBENS), Département de biologie, École normale supérieure, CNRS, INSERM, Université PSL, 75005 Paris, France <sup>2</sup>Université Paris Est-Créteil (UPEC), Faculté des Sciences et Technologie, 61, avenue du Général De Gaulle, 94000 Créteil, France \*debit@bio.ens.psl.eu Longtime considered as transcriptional noise, long noncoding RNAs (lncRNAs) are emerging as central, regulatory molecules in a multitude of eukaryotic species, from plants to animals to fungi. Yet, our knowledge about the occurrence of these molecules in the marine environment, namely in planktonic protists, is still extremely elusive. To fill that gap of knowledge we developed LncPlankton V1.0, which is the first comprehensive database of marine planktonic lncRNAs. By integrating the predictions derived from ten distinctive coding potential tools in a majority voting setting, we identified over 3 million lncRNAs distributed across 418 marine planktonic species from over 9 different phyla. A user-friendly, open-access web interface for the exploration of the database was implemented (https://www.lncplankton.bio.ens.psl.eu/). We believe LncPlankton V1.0 will serve as a rich resource of lncRNAs that will contribute to small- and large-scale studies in a wide range of marine planktonic species and allow comparative analysis well beyond the marine environment.

#### The sophisticated network of histone chaperones is conserved in the pennate diatom *Phaeodactylum tricornutum*

Duc, C.\*, Plantier, V., Rousselot, E. & Tirichine, L.

Nantes Université, CNRS, US2B, UMR 6286, F-44000 Nantes, France \* celine.duc@univ-nantes.fr

DNA is densely packed in the chromatin located within the nucleus. This nucleoproteic structure is notably composed of histones proteins. The histone core is constituted of a H3/H4 tetramer flanked by two H2A/H2B dimers, with an additional H1 linker histone present in chromatin. Histones are highly conserved basic proteins. To prevent spurious interaction within the cell, they are always associated with proteins called the histone chaperones. These proteins ensure histone folding within the cytoplasm, histone nuclear import and incorporation into chromatin. Histones can also be stored by their chaperones in the so-called histone soluble pool. Indeed, during processes operating on DNA such as replication or repair, histones are evicted from chromatin and stored before being recycled and re-incorporated into chromatin. Classification of histone chaperones is based on either the histone they bind or their involvement in processes such as replication, repair or transcription.

In the present study, we retrieved chaperone protein sequences from brown, red and green algae as well as from plants, mycetozoans, animals, ciliates and fungi. We then chose to focus on the unicellular pennate diatom *Phaeodactylum tricornutum* since it displays a small, sequenced and well assembled and annotated genome. We analysed the conservation of the protein domains and motifs involved in chaperone-chaperone and chaperone-histone interactions to determine to which extent they are conserved in this pennate diatom and if new domains can be revealed.

#### Novel Plastome Assembly and Annotation of Florideophyceae

Freiler, M.<sup>1</sup>\* & Lopez-Bautista, J.<sup>2</sup>

<sup>1,2</sup>*The University of Alabama, The United States of America* \*mnfreiler@crimson.ua.edu

Rapidly falling sequencing costs have resulted in the production of an immense amount of raw read data, which is often not used to its full capacity. Here we employ a collection of bioinformatics tools on The University of Alabama High-Performance Computing Cluster (UAHPC) to assemble, annotate and analyze sequences. Our data consists of raw Illumina reads. 101 base pairs in length. Reads were examined with FastQC v0.11.5 before trimming and cleaning by Trimmomatic v0.4. Normalization of

overrepresented sequences to 100x coverage was done by BBNorm. Assembly was performed by SPAdes v3.14; followed by BLASTn 2.9.0, which used a database of previously published Rhodophyta chloroplast genes and genomes to identify the matching scaffold(s). Fast-Plast v1.2.9, which employs Bowtie2 and Jellyfish, was used for coverage analysis and verification assembly. Prior to manual annotation in Geneious 2023.04, protein coding sequences as well as ribosomal genes and tRNA were detected using Plastid Genome Annotator, Chlorobox GeSeq, and tRNAscan-SE 2.0.

We were able to complete de novo assemblies of multiple red algal plastid genomes including *Melanthalia abscissa* and *Polyopes polyideoides*. These preliminary results show that quality assemblies can still be extracted from dated short-read sequence data.

### Nuclear and mitochondrial genetic clines in a red alga presenting multiple transition zones

Guillemin, M-L.<sup>1,2,3</sup>\*, Quesada-Calderón, S.<sup>4,5</sup> & Saenz-Aguledo, P.<sup>1,5,6</sup>

<sup>1</sup>Universidad Austral de Chile, Roscoff, France
<sup>2</sup>Universidad Austral de Chile, Valdivia, Chile
<sup>3</sup>Centro FONDAP de (IDEAL), Valdivia, Chile
<sup>4</sup>AUSTRAL-omics, Vicerrectoría de Investigación, Desarrollo y Creación Artística, Universidad Austral de Chile, Valdivia, Chile.
<sup>5</sup>Instituto de Ciencias Ambientales y Evolutivas, Universidad Austral de Chile, Valdivia, Chile
<sup>6</sup>Las Cruces, Chile.
\*marielaure.guillemin@gmail.com

In the morphospecies Mazzaella laminarioides, a red alga commonly encountered along Chilean rocky coasts, three genetic lineages, named North, Center and South and presenting distinct levels of genetic divergence have been previously described based on sequences of rbcL and COI. Here we present the results obtained for six nuclear microsatellites and the COI acquired for 29 localities positioned between the 28°S and the 43°S, allowing us to study the position, amplitude, and permeability of the contact zones between the three lineages for the first time. The transition zones between incipient sibling species can be pinpointed to a narrow transition zone between North and Center at 32°48'S. At the same time, a mosaic of Center and South sampling sites is observed in an area extending over some 225 kilometers between 37°S and 38°S. None of the four transition zones include sites that can be defined as a true contact zone, an area where two genetic groups are found in sympatry, and population admixture estimated with STRUCTURE detected only a few introgressed individuals even when genetic groups are located a few kilometers apart. Geographic cline analyses reveal steep genotypic clines, especially between the North and Center genetic groups, not necessarily centered on physical dispersal barriers as large estuaries or sandy beaches. We propose that the 32°48'S transition zone represents a historical genetic break maintained by contemporary hydrodynamic conditions. In contrast, the mosaic Center-Sur area at 37°S-38°S could be the product of earthquakes, coastal uplift, and subsequent recolonization events.

### Polysaccharides from the extracellular matrix in brown algae: origins and evolutive histories

Mazéas, L.<sup>1</sup>, Bouguerba, A.<sup>2</sup>, Barbeyron, T.<sup>1</sup>, Brillet-Guéguen, L.<sup>2</sup>, Corre, E.<sup>2</sup>, Cock, M.<sup>1</sup>, Terrapon, N.<sup>3</sup> & Hervé, C.<sup>1\*</sup>

<sup>1</sup>Sorbonne Université Paris 06, CNRS, UMR 8227, Integrative Biology of Marine Models, Station Biologique de Roscoff, Roscoff, France <sup>2</sup>Sorbonne Université Paris 06, CNRS, FR 2424 ABiMS facility, Station Biologique de Roscoff, Roscoff, France <sup>3</sup>CNRS, Aix Marseille Univ, UMR 7257 AFMB, 13288 Marseille, France \*cecile.herve@sb-roscoff.fr

Brown algae are phylogenetically as distant from plants as they are from other multicellular organisms. They belong to the Stramenopiles, which also comprises diatoms, oomycetes, etc. The extracellular matrix (ECM) surrounding brown algal cells play key roles in development, cell signaling and defense responses. It has a biochemistry based on the gel-forming alginates and the fucose-containing sulfated polysaccharides (FCSPs). The lack of genomic data in brown algae was impairing functional elucidations. The *Ectocarpus* genome was obtained in 2010 and our efforts have now been extended with the sequencing of 40 brown algal genomes and sister species. Our analysis of the Carbohydrate Active Enzymes (CAZymes) indicates that they do not harbor gene expansion in relation to tissue complexity. Along with the alginate biosynthetic route, a degradation pathway is present. Both pathways may have raised in the sister species Schizocladia, before the divergence of brown algae, and through the acquisition of bacterial genes. Additionally, genes for the synthesis of FCSPs are predicted. Our investigation provides a comprehensive understanding of the origins and evolution of the metabolic pathways of ECM polysaccharides in brown algae. It also opens up possibilities for functional studies of ECMgenes in these organisms.

#### What make *Phaeocystis* species putatively good symbiont for photosymbiosis?

Juery, C.1\*, Blanc-Mathieu, R., Allen, A., Füssy, Z. & Decelle, J.<sup>2</sup>

<sup>1,2</sup>Cell & Plant Physiology Laboratory, UMR 5168 CEA-CNRS-Univ. Grenoble Alpes – UMR1417 INRAE, Grenoble, France; \*correling inerv@cea fr

\*caroline.juery@cea.fr

Photosymbiosis is a key type of symbiosis to explore the genomic co-evolution of two partners that can lead to the complete integration of plastids. *Phaeocystis* represent a microalgae genus widespread in all oceans of the globe that is involved in two types of photosymbiosis: the entire algae with the Acantharae group of radiolarians (Decelle *et al.* 2012, Uwizeye *et al.* 2021) and only the plastids engulfed by a Dinoflagellate through kleptoplastidy (Hehenberger *et al.* 2019). For the latest, horizontally transferred genes form the kleptoplast to the host cell nucleus have been identified but for the former, no genomic evidence of partners genomic co-evolution has been demonstrated so far. In our study, we aim at understanding what make *Phaeocystis* species a good potential symbiont at the genomic level inspecting its sets of transporter genes.

For this, we studied the transporter genes of 7 haptophytes including three species of *Phaeocystis*. Thought phylogenetic and protein motifs conservation analysis, we discover three families of transporter genes (sugar transport) that present a specific evolutionary fate within *Phaeocystis* species. We also demonstrate that supplementary gene duplications of Triose Phasphate Transporters (TPT) in *Phaeocystis antarctica* compare to the two other close relative species might be one of the major factor that could explain its involvement in the kleptoplastidic relation.

Our study is first step to decipher the genomic and genetic evolution of protists through photosymbiotic interactions that represent a crucial element to understand why the ocean is red and the land green (Falkowski PG 2004).

### Rates of evolution in chloroplast genes within the only freshwater species of the Bangiales, *Bangia atropurpurea*

Poletto Borges, V., Witt, J.D.S. & Müller, K.M.\*

Department of Biology, University of Waterloo, Waterloo, Ontario, Canada. \*kmmuller@uwaterloo.ca

The filamentous red algae genus *Bangia* (Bangiales) has been observed in both marine and in freshwater habitats and is a known invader in the North American Laurentian Great Lakes. Interestingly, the freshwater species, *B. atropurpurea*, is more closely related to species within the genus *Porphyra* than to the marine filamentous forms of *Bangia fuscopurpurea*. *Bangia atropurpurea* is also the only species within the Bangiales to occupy freshwater habitats and appears to be strictly asexual. Recently we have completed a draft chloroplast genome of this species that reaffirms the relationship of this species with the genus *Porphyra*. We then used the chloroplast genome of *B. atropurpurea* to calculate the rates of substitution in 71 chloroplast genes, allowing the identification of evolutionary patterns that provide insights into selective forces on genes that may have contributed to the invasion of the Great Lakes. Chloroplast gene sequences were compared with genes from 29 Rhodophyta species originally isolated from marine, freshwater, salt marshes and hot springs. This extensive analysis revealed trends in substitution rates according to taxonomic groups but also by habitat, where the Bangiales exhibited the most diverse rates for the *atp, psa, rps* and *rpl* gene families. Moreover, the substitution rates indicated that within the Bangiales, *B. atropurpurea* is distinct, with rates that contradict trends observed in the other Bangiales species.

#### A comprehensive study of histone proteins in brown algae

Rousselot, E.\*, Tirichine, L. & Duc, C.

Nantes Université, CNRS, US2B, UMR 6286, F-44000 Nantes, France \*ellyn.rousselot@univ-nantes.fr

The nucleosome is the subunit of chromatin and is composed of 147bp of DNA wrapped around a histone core (a H3/H4 tetramer flanked by two H2A/H2B dimers). There is a fifth histone, the H1 linker that binds to the nucleosome and linker DNA, located in between two nucleosomes. Histones are small basic proteins; the histone core presents a strong sequence conservation within eukaryotes while H1 is much more divergent. Histones harbor several isoforms and canonical histones are expressed only during S-phase of the cell cycle while variants are expressed throughout the cell cycle.

In the present study, we carried out a detailed analysis of histone protein sequences in publically available genomes of 20 brown algae such as the unicellular pennate diatom *Phaeodactylum tricornutum*. We also included some species of green and red algae, plants, mycetozoans, animals, ciliates and fungi. We notably observed that in brown algae H3.1 and H3.3 isoforms differ by an AT/TA motif at positions 31-32.

In animals, canonical histone transcripts have a stem loop structure while variant histone transcripts are polyadenylated. A specific 3'end processing machinery ensures cleavage of canonical histone mRNAs. This machinery was thought to be restricted to metazoans and green algae and it was documented as absent in a majority of plant species. We examine the presence of the various components of this machinery in brown algae.

# Genome wide assessment of genetic diversity and transcript variations in 17 accessions of the model diatom *Phaeodactylum tricornutum*

Yang, F.<sup>1¥</sup>, Chaumier, T.<sup>1¥</sup>, Manirakiza, E.<sup>1</sup>, Wu, Y.<sup>1</sup>, Chandola, U.<sup>1</sup>, Jesus, B.<sup>2</sup>, Piganeau, G.<sup>3</sup>, Groisillier, A.<sup>1</sup> & Tirichine, L.<sup>1\*</sup>

<sup>1</sup>Nantes Université, CNRS, US2B, UMR 6286, F-44000 Nantes, France <sup>2</sup>EA2160, Laboratoire Mer Molécules Santé, Université de Nantes, Nantes, France <sup>3</sup>Integrative Biology of Marine Organisms (BIOM), Sorbonne University, CNRS, Oceanological Observatory of Banyuls, Banyuls-sur-Mer, France ¥ Equal contribution

Diatoms are recognized as highly diverse and prevalent phytoplankton in the ocean, contributing significantly to the global carbon cycle and supporting marine ecosystems. Among diatoms, *Phaeodactylum tricornutum* is widely used as a model species to understand the biology of this important group of phytoplankton. Seventeen natural accessions of this species including old and more recently sampled variants, were collected from different locations in the world's oceans and sequenced, representing a valuable resource for understanding how diatoms respond and adapt to their environments. In this study, with the whole genome sequencing including Illumina and Pac Bio, we drew a complete and comprehensive landscape of the genomic and transcriptomic diversity within the species revealing new clades and novel transcripts and revisiting previously defined ecological niches. The details of the study are discussed.

#### SYMPOSIUM 04 "Algae and their intimate partners"

### Searching *Phaeobacter* spp. in *Ulva* spp. microbiota: a metadataanalysis

Bruni, L.<sup>1\*</sup>, Pereira, M.<sup>1</sup>, Del Olmo, G.<sup>1</sup>, Nappi, J.<sup>2</sup> & Pintado, J.<sup>1</sup>

<sup>1</sup>Marine Ecology and Resources Group. Institute of Marine Research (IIM-CSIC), Galicia, Spain. <sup>2</sup>Centre for Marine Science and Innovation. School of Biological, Earth and Environmental Sciences. The University of New South Wales, Sydney, Australia. \*lbruni@iim.csic.es

*Phaeobacter* spp. constitute bacterioplankton and form biofilms on abiotic and biotic surfaces, as macroalgae. They are able to produce tropodithietic acid, antibiotic active against other marine bacteria (e.g. *Vibrio* spp.), thus potentially playing a role in bacterial interactions in algal biofilms. *Phaeobacter* spp. have been isolated from *Ulva* spp., and used in experimental colonisation of *U. ohnoi*, providing biological control of pathogenic *Vibrio* in fish-*Ulva* integrated multitrophic recirculating aquaculture systems (IMTA-RAS). Knowing *Phaeobacter* presence in different *Ulva* species is of great interest, however, metadata analyses of *Phaeobacter* distribution on macroalgae has not been done.

By mining available genomic information, we aim to determine the presence, distribution, and relative abundance of *Phaeobacter* in *Ulva* microbiota, and to have hints at the conditions enabling the bacterium to thrive. Results from a study we have conducted showed that *Phaeobacter* was detected in *U. australis* 

and *U. rigida* from two different rock pools in Tragove, Galicia, Spain, with different frequency depending on the environment. When present, *Phaeobacter* did not exceed 0.3% relative abundance. Those results are being compared with the ones published, retrieving 16S rRNA gene sequencing data of *Ulva* microbiota and classifying the ASVs using the BLCA method. *Phaeobacter* distribution and relative abundance in the different *Ulva* species will be assessed, and the available information of the biotic and abiotic factors which could affect the presence of *Phaeobacter* will be analysed. The overarching goal is to increase the knowledge of the relationship between *Phaeobacter* and *Ulva* to improve the understanding of its ecology, which would base biological control strategies in aquaculture.

### Quantitative study of the microbial communities living on the surface of the green seaweed *Ulva sp.*

Burlot, A-S.<sup>1\*</sup>, Guillouche, C.<sup>1</sup>, Cadieu, S.<sup>1</sup>, Paulino-Le-Deun, S.<sup>1,2</sup>, Lasserre, B.<sup>3</sup>, Gervois, A.<sup>3</sup>, Huchette, S.<sup>4</sup>, Rieusset, L.<sup>1</sup>, Bedoux, G.<sup>1</sup> & Bourgougnon N.<sup>1</sup>

<sup>1</sup>Laboratoire de Biotechnologie Et Chimie Marines, EMR CNRS 6076, UBS, IUEM, 56000 Vannes, France <sup>2</sup>Laboratoire des Sciences de l'Environnement Marin, UMR 6539 UBO/CNRS/IRD/IFREMER, 29280 Plouzané, France

<sup>3</sup>Faculté des Sciences et Sciences de l'Ingénieur, Département Sciences et Vie de la Terre, UBS, 56000 Vannes, France

<sup>4</sup>*France Haliotis SCEA, Brest, France* \*anne-sophie.burlot@univ-ubs.fr

In marine environment, any submerged surface is quickly colonized by microorganisms (e.g., bacteria, fungi, viruses and microalgae), leading to the formation of biofilms. Macroalgae are no exception to this phenomenon. Fixed, they present on their surface communities of epibiotic microorganisms. These communities play an important ecological role in certain biotic interactions, such as the protection of surfaces against biological fouling and proliferation of pathogenic agents, their ingestion by herbivores and their ability to resist xenobiotics. They are also involved in the seaweed growth and development. This host-microbiota or "holobiont" regulates the relationship between the algae-basibiont and its epibiont. It can therefore be assumed that environmental stress related to modifications caused by eutrophication and climate change could disrupt these complex mutualistic relationships conducting to the degradation of coastal marine ecosystems.

The objective of this study is to measure the impact of environmental factors on the physiology of *Ulva sp.* and on the associated microbial density.

*Ulva sp.* were harvested at different places on the same day; from protected area to stranding biomass and also from seaweed farming tanks. Microorganisms were dissociated from recovered biofilm on seaweed surface. Microbial density was measured by flow cytometer and several populations of bacteria and eucaryotic cells were discriminated and observed by scanning electron and confocal microscopy.

Different populations of bacteria and microalgae were detected. Moreover, some environmental factors like seawater salinity and temperature have shown their influence on the microbial communities' distribution on *Ulva sp.* 

### Does light affect the presence of *Phaeobacter* in the *Ulva ohnoi* holobiont by modifying bacterial communities?

Del Olmo, G.<sup>1</sup>\*, Ruiz, P.<sup>1</sup>, Nappi, J.<sup>2</sup>, Thomas, T.<sup>2</sup>, Egan, S.<sup>2</sup>, Cremades, J.<sup>3</sup> & Pintado, J.<sup>1</sup>

<sup>1</sup>Marine Ecology and Resources Group. Institute of Marine Research (IIM-CSIC), Galicia, Spain. <sup>2</sup>Centre for Marine Science and Innovation. School of Biological, Earth and Environmental Sciences. The University of New South Wales, Sydney, Australia.

<sup>3</sup>Coastal Biology Research Group (BioCost). CICA - Interdisciplinary Center for Chemistry and Biology, University of A Coruña, Galicia, Spain.

\*gdelolmo@iim.csic.es.

Ulva is used in integrated recirculating multi-trophic aquaculture recirculation systems (IMTA-RAS) due to its biofiltration capacity. Moreover, Ulva provides a niche for biofilmforming bacteria such as Phaeobacter sp. with the ability to antagonize fish pathogens such as Vibrio anguillarum. Phaeobacter colonization on Ulva can be used as a pathogen control strategy in IMTA-RAS. However, an experiment conducted in multi-well plates under different light intensities and with/without Phaeobacter inoculation showed that high lightintensity negatively affected the maintenance of *Phaeobacter* biofilms on *Ulva* surface after 14 days. Nevertheless, this did not happen when *Phaeobacter* was colonizing inert surfaces. We hypothesized that the reduction of *Phaeobacter* could be due to changes in the bacterial communities promoted by high light intensity. The microbial samples of algal surface from the above-mentioned experiment were used. The 16S rRNA gene was sequenced to study the bacterial composition in U. ohnoi under the different conditions at days 7 and 14. Results indicated that light had no significant influence on the Ulva bacterial communities, and the experimental colonization by Phaeobacter only seemed to significantly modify the communities at day 7, but not at day 14. Some ASVs were found to be significantly affected by light intensity, such as ASV6 (Alteromonas) which was more abundant in exposed light samples, and treatment, such as ASV10 (Litorimonas) whose relative abundance was higher in samples with Phaeobacter inoculation. In conclusion, reduction of Phaeobacter is not explained by the general composition of Ulva bacterial communities in different light intensity and could be influenced by other Ulva holobiont elements.

#### Chemical exploration of the Ascophyllum nodosum holobiont

Demoulinger, G.<sup>1,2</sup>, Le Guillard, C.<sup>2</sup> & Prado, S.<sup>1</sup>

<sup>1</sup>Unité Molécules de Communication et Adaptation des Micro-Organismes (UMR 7245), Muséum National d'Histoire Naturelle, CNRS, Sorbonne Université, Paris, France <sup>2</sup>Plant Nutrition Department, Agro Innovation International, Timac Agro, Saint-Malo, France

Among the algal products developed to alleviate abiotic and biotic stresses in plants, many contain extracts of brown algae, and *Ascophyllum nodosum* is one of the main species exploited. However, the chemical nature of the bioactive compounds present in *A. nodosum* extracts has not been full characterised, even with regard to secondary metabolites.

According to the recent literature, seaweeds live in close relationship with endophytic and epiphytic microorganisms, forming with their host algae a functional dynamic entity called an holobiont. Within this holobiont, the microorganisms and their host produce secondary metabolites that are often involved in the health and stability of this entity, raising questions about the biosynthetic origin of the compounds found in *A. nodosum* extracts.

Thus, in order to understand the entire metabolome of *A. nodosum* extracts, we isolated and characterised the cultivable microbiota associated with the brown alga and developed chemical dereplication based on molecular networking from LC-MS and LC-MS/MS data.

This work is also part of the ANR SEABIOZ project.

#### The bacteriome of *Porphyra umbilicalis* in the North-Atlantic across time and space

Engelen, A.H<sup>\*1</sup>, Morrison, H.G.<sup>2</sup>, Capistrant-Fossa, K.A.<sup>3</sup>, Quigley, C.T.C.<sup>3</sup>, Aydlett, M.<sup>3</sup>, Serrão, E.-A.<sup>3</sup>, Brodie, J.<sup>4</sup>, Gachon, C.M.<sup>5</sup>, Badis, Y.<sup>5</sup>, Johnson, E.E.<sup>6</sup>, Hoarau, G.<sup>7</sup>, Abreu, M.H.<sup>8</sup>, Stearns, L.A.<sup>9</sup> & Brawley, S.H.<sup>7</sup>

<sup>1</sup>Centro de Ciências do Mar, Portugal.
<sup>2</sup>Marine Biological Laboratory, Josephine Bay Paul Center, USA.
<sup>3</sup>School of Marine Sciences, University of Maine, USA.
<sup>4</sup>Natural History Museum, Department of Life Sciences, UK.
<sup>5</sup>Scottish Association for Marine Science, UK.
<sup>6</sup>Département de Biologie, Université Laval, Canada.
<sup>7</sup>Faculty of Biosciences and Aquaculture, Nord University, Norway.
<sup>8</sup>ALGAplus, Portugal.
<sup>9</sup>Department of Geology, University of Kansas, USA.
\*aengelen@ualg.pt

The microbiome of foundation brown seaweed Fucus vesiculosus has been shown to be strongly influenced by tissue specificity and latitudinal gradients across the North-Atlantic. In the same intertidal zone, the commercially important Porphyra species can also be found. Previous research in Schoodic, Maine has demonstrated that the bacterial genus *Lewinella* is consistently and abundantly present on blades of Porphyra. In addition, members of the Bacteroidetes, Proteobacteria, and Planctomycetes, which are known to digest the galactan sulfates of red algal cell walls, are well-represented. Isolated members of the Planctomycetes from Porphyra have been found to contain various genes encoding selenoproteins, many of which are enzymes that ameliorate the impact of environmental stresses that occur in the intertidal habitat. Holdfasts have been shown to be enriched in Planctomycetes and Alphaproteobacteria, particularly the possible symbiont Sulfitobacter. Despite the commercial importance of *Porphyra* and the availability of its genome, little is known about its microbiome across spatial and temporal scales. In this study, we investigated the bacteriomes of Porphyra umbilicalis blades and holdfasts across their North-Atlantic distribution range in three years and two seasons to identify microbiome drivers and test whether local findings persist at larger spatial and temporal scales, by analyzing the partial 16S rRNA gene of ~700 specimen from nine sites covering three host species, three years, and two seasons.

#### Investigating bacterial diversity of the ulva holobiont in industrial cultures

Estoup, P.<sup>1,2</sup>\*, Gernigon, V.<sup>3</sup>, Raimbault, P.<sup>1</sup>, Avouac, A.<sup>2</sup>, Blanc, G.<sup>1</sup> & Gobet, A.<sup>2</sup>

<sup>1</sup>*MIO, Aix Marseille Université, Université de Toulon, CNRS, IRD, UM 110, Marseille, France* <sup>2</sup>*MARBEC, Univ Montpellier, CNRS, Ifremer, IRD, Sète, France* <sup>3</sup>*Eranova, Port-Saint-Louis-du-Rhône, France* \*paul.estoup@univ-amu.fr

In the macroalgal holobiont, interactions between the host and its associated microbiome play a key role in host growth and productivity. Ulva's bacterial microbiome is known to act on host morphology and physiology. Ulva is commonly known for causing green tides due to eutrophication, but fluctuations of its microbiome in these conditions are poorly known. Our study aims to describe and follow the bacterial diversity associated with ulva industrial cultivation, with (enriched, ENR) and without (seawater only, SW) nitrate-based fertilization. From March to May 2021, cultivation water and ulva thallus were collected in triplicate weekly and bimonthly, respectively. The bacterial community from ulva's biofilms and water was assessed by metabarcoding of the 16S rRNA gene. In the water or the biofilm, microbial communities followed distinct ecological patterns in the SW and ENR conditions, and fluctuated with temperature (from 6 °C to 18 °C). For instance, ASVs identified as *Granulosicoccus*, known as a genus of potential algal polysaccharide degraders, was amongst the most abundant in the biofilms and decreased from 78.1% to 28.5% and from 45.0% to 1.2%, in SW and ENR, respectively. Interestingly, the ENR condition seemed to have enriched genera known for their role in ulva morphogenesis: *Maribacter, Roseobacter* and *Sulfitobacter*. Fertilization thus led to a shift in the bacterial community composition and structure associated with ulva. This study gives an overview of the effect of large inputs of agricultural fertilizers on ulva's associated microbiome in a cultivation basin and could be extrapolated to coastal green tides.

#### Marine microbial protein-carbohydrate interactions with red algal molecules

Ficko-Blean, E.\*<sup>1</sup>, Stubbs, K.<sup>2</sup>, Wallace, M.<sup>2</sup>, Roret, T.<sup>3</sup>, Thomas, F.<sup>1</sup>, Guée, L.<sup>1</sup>, Czjzek, M.<sup>1</sup>, Fanuel, M.<sup>4,5</sup>, Ropartz, D.<sup>4,5</sup> & Michel, G.<sup>1</sup>

<sup>1</sup>CNRS, Integrative Biology of Marine Models (LBI2M), Station Biologique de Roscoff (SBR), Sorbonne Université, Roscoff, France
 <sup>2</sup>School of Molecular Sciences, The University of Western Australia, Crawley, WA 6009, Australia
 <sup>3</sup>CNRS, FR 2424, Station Biologique de Roscoff, Sorbonne Université, Roscoff, France
 <sup>4</sup>INRAE, UR BIA, Nantes, France
 <sup>5</sup>INRAE, BIBS Facility, Nantes, France
 \*eficko-blean@sb-roscoff.fr

Macroalgae are important primary producers from the marine ecosystem and a large proportion of their organic biomass, mainly polysaccharides, is recycled through the marine food chain. Carrageenans are a major component of the cell wall of red macroalgae, they are sulfated and extremely complex. Marine microorganisms, such as the model bacterium *Zobellia galactanivorans* Dsij<sup>T</sup>, are key players in the recycling of organic matter in the oceans. In order to process carrageenan, *Zobellia* produces highly specific enzymes coded within a multifaceted carrageenan-induced regulon, including a non-canonical carrageenan-utilization locus (CarPUL). Within the CarPUL are genes that encode the recently discovered *exo*-( $\alpha$ -1,3)-3,6-anhydro-Dgalactosidase activity. These enzymes have exquisite specificity for the bicyclic and highly sterically restrained carrageenan monosaccharide 3,6-anhydro-D-galactose found only in red algae. Here, we examine the fine structure and function of the bacterial *exo*-( $\alpha$ -1,3)-3,6-anhydro-D-galactosidases using a combination of novel chemical tools and fine structural analyses.

#### Role of bacteria in the allelopathic ability of an autotrophic biofilm

Avouac, A.<sup>1</sup>, Perez, S.<sup>1</sup>, Le Floc'h, E.<sup>1</sup>, Félix, C.<sup>1</sup>, Guihéneuf, F.<sup>2</sup>, Leboulanger, C.<sup>1</sup>, Gobet, A.<sup>1</sup> & Fouilland, E.<sup>1\*</sup>

<sup>1</sup>MARBEC Univ Montpellier, CNRS, IRD, IFREMER, France <sup>2</sup>INALVE, France \*Eric.Fouilland@cnrs.fr Allelopathy is defined as the inhibition of competitor organisms by chemical means, allowing one species to alter the ability of another species to grow and exploit resources. Most studies on allelopathic interactions between autotrophic microorganisms and/or bacteria have focused on free-living species in water, and very few studies have been conducted on microalgae forming biofilms. However, in shallow and low-flowing waters where autotrophic biofilms can reach high levels of biomass and productivity, it has been suggested that the amount of allelopathic products released by the biofilm could be sufficient to inhibit surrounding planktonic organisms.

We have developed and applied a protocol to detect the inhibitory activity of a *Tetraselmis suecica*grown biofilm with its associated microbiome under semi-controlled growth conditions. We were able to demonstrate that this biofilm had the ability to inhibit a target bacterial strain *Alteromonas* sp. (originally isolated from the microalga *Isochrysis galbana*). The measured inhibition was found to be even more intense than that of a control Amoxicillin antibiotic (+10 to 50%).

Furthermore, we isolated a bacterial strain from the biofilm, whose 16S rDNA sequence was similar to *Vibrio* sp., which showed the ability to inhibit not only the *Alteromonas* sp. but also a bacterial strain, whose 16S rDNA sequence was similar to *Pseudoalteromonas* sp., isolated from the environment (i.e. Thau Lagoon, Mediterranean coast, France). This suggests that bacterial species growing in the *Tetraselmis suecica* biofilm may play an important role in the allelopathic ability of the whole biofilm.

#### Production of fatty acids rich in DHA and EPA from microalgae to enrich infant growth milk

Mir, Y. & Lbouhmadi, R.\*

Faculty of Medicine and Pharmacy, University of Ibn Zohr, Agadir, Morocco \*rkia6lbouhmadi@gmail.com

Fatty acids such as DocosaHexaenoic Acid (DHA) and EicosaPentaenoic Acid (EPA) are of growing interest for their positive impact on human health. Coming mainly from marine sources such as fish, these two compounds are facing supply problems due to overfishing.

The objective of this project is to develop a robust, cold process technique to extract DHA and EPA from microalgae biomass in order to use them as an additive in growing-up milk for infants.

Three different strains were selected for this study; *Parachlorella kessleri* (GEPEA UMRCNRS6144, University of Nantes) and *Cyclotella spp* and *Scenedesmus spp* (collected from the region of Agadir, Morocco).

The effect of several culture conditions and their impact on EPA and DHA accumulation was studied. The lipids composition of the tree strains was analyzed using GC-MS.

Results indicated that culture conditions such as sodium bicarbonate concentration, light intensity and cultivation time affected biomass and fatty acides production. GC-MS results showed 40% increase in Polyunsaturated Fatty Acids compared to Saturated Fatty Acids under specific stress conditions with 20% increase of EPA amount especially for *Cyclotella spp*.

This study confirms that the productivity of lipids can be increased by stress factors and *Cyclotella spp*. strains can be considered as a potential candidate for DHA and EPA production.

### Toward an Antarctic phycopathology: description of natural pathosystems associated to the green alga *Urospora penicilliformis*

Murúa, P.<sup>1,2,\*</sup>, Muñoz, L.<sup>1,3</sup>, Añasco, B.<sup>1</sup>, Badis, Y.<sup>4</sup>, van West, P.<sup>3</sup> & Gachon, C.M.M.<sup>5</sup>

<sup>1</sup>Laboratorio de Cultivo de Macroalgas y Ficopatología FICOPAT, Instituto de Acuicultura, Universidad Austral de Chile, Sede Puerto Montt.

<sup>2</sup>The Scottish Association for Marine Science, Scottish Marine Institute, Culture Collection for Algae and Protozoa, Oban, PA37 1QA, United Kingdom.

<sup>3</sup>Aberdeen Oomycete Laboratory, International Centre for Aquaculture Research and Development, University of Aberdeen, Foresterhill, Aberdeen, AB25 2ZD, United Kingdom.

<sup>4</sup>UMR 8227, Station Biologique, Place Georges Teissier, Roscoff, 29680, France

<sup>5</sup>Muséum National d'Histoire Naturelle, CNRS CP 26, 57 rue Cuvier, 75005 Paris, France

\*pedro.murua@uach.cl

Antarctic biodiversity is one of the most unknown in the world, a situation that increases with less charismatic organisms, such as algal pathogens. Antarctic phycopathology is virtually non-existent, both in biodiversity and in the availability of laboratory pathosystems. In this work we report what would be the first record of an Antarctic marine oomycete, which infects the green alga Urospora penicilliformis. The pathogen is an obligate intracellular holocarpic parasite with an "olpidioid" development, which is reminiscent of "primitive" Peronosporomycete omycetes. The pathogen was found in all the sampled areas around King George and Nelson Islands, in two consecutive years (20222023), but with very variable prevalence. In a follow-up sampling within a population near the Escudero base (Bahía Fildes), it was determined that there were no differences between central or marginal areas for outbreaks. These outbreaks would be regulated by an extraordinary ability of Urospora to generate propagules and recruit healthy individuals within its distribution, which would help to keep the disease at low levels. As a second natural pathogen we also describe a pathogen (Gammaproteobacteria) that colonizes Urospora not only from Antarctica, but also from sub-Antarctic environments (e.g. Los Lagos). The pathogen grows colonially, digests the host's cell wall, and forces the host's cytoplasm out of the cell, to be digested by the bacterial cluster. Marker-assisted taxonomy for both organisms revealed undescribed lineages, potentially new species to be described.

### Characterization of Cell Cycle Events Affected by Light dose in the microalgae *Tisochrysis lutea*

Pageault, L.<sup>1\*</sup>, Stachowski-Haberkorn, S.<sup>2</sup>, Charrier, A.<sup>2</sup>, Mairet, F.<sup>2</sup>, Garnier, M.<sup>1</sup>, Carrier, G.<sup>1</sup>, Bérard, J-B.<sup>2</sup>, Bougaran, G.<sup>2</sup>, Saint-Jean, B.<sup>1</sup>

<sup>1</sup>GenAlg, PHYTOX unit, IFREMER France <sup>2</sup>PhysAlg, PHYTOX unit, IFREMER France \*Laura.pageault@ifremer.fr

The interest of the exploitation of the microalgae *Tisochrysis lutea* in aquaculture or its biotechnological potential in comestic, pharmaceutical or health industry is no longer to be demonstrated. While the physiology and the metabolism of this microalgae has been intensively studied, many questions still remain unanswered such as the mechanisms of regulation of the cell cycle. Here we present a first description of the cell cycle depending on the length of the light period. We considered *T. lutea* cell cycle into interdivision (light period) and division phases (dark period) on the basis of changes in cell

concentration and diameter and found the existence of a minimum threshold dose of light necessary for the induction of cell division. Also, our first results suggest the existence of a 'commitment sizer' which controls that cells have exceeded the minimum cell size required to initiate cell division.

A better knowledge of all the prerequisites for the passage of the commitment point also informs us about the conditions favoring/inducing these mitosis phenomena, closely related to meiotic events that can testify to the ability of *T. lutea* to sexually reproduce. The demonstration of sexual reproduction in this species would open new opportunities to use hybridization for genetic improvement programs.

#### ROS is important in the life cycle and during infection of *Pythium porphyrae,* the causative agent of red rot disease

Park, H.\*, Shim, E. & Kim, G.H.

Department of Biological Sciences, Kongju National University, Gongju 32588, Korea \* gksk9063@smail.kongju.ac.kr

Reactive oxygen species (ROS) are important signaling molecules that regulate cellular physiological responses in many organisms, but little is known about how ROS signaling is involved in the life cycle of marine oomycetes. The oomycete Pythium porphyrae is the causative agent of red rot disease, which causes severe damage to Pvropia sea farms, and while much research has been done to mitigate the damage, there is still a lack of understanding of the signals that regulate its life cycle or its infection processes in the host. We divided the life cycle of Pythium porphyrae into three stages and examined the production of ROS using DCFH-DA staining. During sexual reproduction ROS accumulation was observed during oogonium development, antheridium formation and fusion with the oogonium, but ROS disappeared at the end of fertilization. If multiple antheridia bound to the oogonium, the successful antheridium stopped ROS production, but the unsuccessful antheridia continued to produce ROS. In asexual reproduction ROS accumulates in large amounts during zoosporangium formation, but once zoospores are released, no more ROS is produced. During infection, active accumulation of ROS can be observed in zoospores of *Pythium porphyrae* attached to the surface of host cells and in hyphal cells within the host. Treatment with DPI, an inhibitor of NADPH oxidase that produces ROS in the cells, decreased fertilization success, hyphae growth, zoosporangium formation, and infection rates. In this study, we identified an important role for ROS, produced by NADPH oxidases, in the life cycle and host infection of Pythium porphyrae.

#### Interactions between Ulva sp. and its cultivable epiphytic bacteria

Paulino, S.<sup>1,2</sup>\*, Le Strat, Y.<sup>2</sup>, Petek, S.<sup>3</sup>, Lebrun, M.<sup>2</sup>, Bourgougnon, N.<sup>1</sup> & Le Blay, G.<sup>2</sup>

<sup>1</sup>UBS, Laboratoire de Biotechnologie et Chimie Marines, EMR CNRS 6076, Vannes, France <sup>2</sup>Univ Brest, CNRS, IRD, Ifremer, LEMAR, F-29280 Plouzane, France <sup>3</sup>IRD, CNRS, Ifremer, Univ Brest, LEMAR, F-29280 Plouzane, France \*sauvann.paulino-le-deun@univ-ubs.fr

The green macroalgae *Ulva* sp., like all submerged surfaces, is colonized by a large variety of microorganisms, among which bacteria seem predominant. Bacterial communities living in ecological niches on the surface of macroalgae are distinct from bacteria present in the surrounding environment. These algal-associated bacteria develop different mechanisms to control the colonization of their habitat by microorganisms, including bacteria. The algal surface can be considered as a reservoir of bacteria

producing molecules with different properties (antibacterial, antibiofilm or probiofilm) potentially valuable in biotechnology. In this study, nearly 70 bacterial strains were isolated from algal surfaces, characterized, sequenced and screened for their antibacterial, antibiofilm or probiofilm activity against model marine bacterial strains in biofilm formation or marine opportunistic pathogen. Several strains showed particularly strong activities and their supernatants were explored by LC-MS/MS technology in order to discriminate the molecules and groups of molecules potentially responsible for the observed activity.

#### Shedding light on the *Ulva* holobiont: the role of light in interactions with *Phaeobacter* bacteria

Pintado, J.<sup>1\*</sup>, Del Olmo, G.<sup>1</sup>, Ruiz, P.<sup>1</sup>, Qui-Minet, Z.N.<sup>1</sup>, Wichard, T.<sup>2</sup>, Egan, S<sup>3</sup>, Thomas, T.<sup>3</sup>, Nappi, J.<sup>3</sup> & Cremades, J<sup>4</sup>

<sup>1</sup>Marine Ecology and Resources Group. Institute of Marine Research (IIM-CSIC), Vigo, Galicia, Spain <sup>2</sup>Jena School for Microbial Communication, Institute for Inorganic and Analytical Chemistry, Friedrich Schiller University Jena, Jena, Germany <sup>3</sup>Centre for Marine Science and Innovation. School of Biological, Earth and Environmental Sciences. The University of New South Wales, Sydney, Australia <sup>4</sup>Coastal Biology Research Group (BioCost). Centro de Investigaciones Científicas Avanzadas (CICA), Universidad de A Coruña, A Coruña, Galicia, Spain \*pintado@iim.csic.es

Natural populations of *Ulva* seem well adapted to conditions of light heterogeneity, and this adaptation could suggest the involvement of microbial interactions. Since the bacterial communities associated with *Ulva* spp. play an important role in morphogenesis and reproduction, as in the adaptation to stress, it is possible that bacteria are also important for *Ulva* spp. adaptation to light conditions. Moreover, *Ulva* spp. laminar thallus provides an important niche for biofilm-forming bacteria, including bacteria with antagonistic activity against other marine bacteria, such as those from the *Phaeobacter* genus that produce tropodithietic acid (TDA). The probiotic effect of *U. ohnoi* experimentally colonized with a *Phaeobacter* strain, previously isolated from *Ulva* spp., was demonstrated in experimental infections with *V. anguillarum*. Those results paved the way to the engineering of *Ulva*associated bacterial communities as strategy for disease control in fish-*Ulva* IMTA-RAS cultures. However, a negative influence of high light and *U. ohnoi* growth on the maintenance of *Phaeobacter* biofilms was observed. This did not happen when *U. ohnoi* was maintained in the dark, with no growth.

Microbial communities and the exo-metabolome of *Ulva* surfaces were analysed to elucidate the underpinning mechanisms in the response to light variables. Light showed a significant effect in the metabolome produced by the *U. ohnoi* holobiont but did not affect significantly bacterial communities. Therefore *Phaeobacter* maintenance and TDA production would be affected by *U. ohnoi* physiology. Adapting *Ulva* cultivation within the IMTA-RAS systems to include light and dark phases, ensured *Phaeobacter* was retained and the probiotic effect promoted.

#### **Seasonal dynamics of macroalgae-associated bacterial communities** Brunet, M., Le Duff, N., Barbeyron, T. & Thomas, F.\*

Sorbonne Université, CNRS, Integrative Biology of Marine Models (LBI2M), Station Biologique de Roscoff (SBR), 29680 Roscoff, France \*fthomas@sb-roscoff.fr

Bacteria and macroalgae are closely associated and their interactions are at the basis of various essential biological processes in coastal oceans. Understanding the drivers of microbial community composition in macroalgae-dominated habitats is then crucial. Although the bacterial community composition of several macroalgae has been addressed over the past decades, comparative studies encompassing different algal species and knowledge on the dynamic of bacterial community succession over large temporal scales remain scarce.

Here, we investigated the seasonal variations of bacterial communities associated with widespread healthy intertidal macroalgae in temperate habitat. The abundance and taxonomic composition of the surface microbiota of brown (*Laminaria digitata*, *Fucus serratus*, *Ascophyllum nodosum*), red (*Palmaria palmata*) and green (*Ulva* sp.) algae were monitored monthly over one year using quantitative PCR and 16S rDNA metabarcoding.

While the bacterial classes *Alphaproteobacteria*, *Gammaproteobacteria* and *Bacteroidia* dominated the microbiome of all algae throughout the year, analyses revealed host-specific bacterial communities at lower taxonomic levels. Seasonal succession patterns were observed with all algae, resulting from the variation in abundance of a limited number of ASV. It was shown that biotic and abiotic environmental parameters - such as temperature, salinity or chlorophyll concentration - likely contribute to these temporal changes, together with algal morphology.

Overall, this study provides deeper insight into macroalgae biofilm composition and fluctuation and ultimately contributes to decipher the factors that shape epiphytic bacterial communities.

#### Highly divergent Picorna- and CRESS-like viruses associated with bleached thalli of the green seaweed *Ulva*

van der Loos, L.M.<sup>1\*</sup>, Willems, A.<sup>2</sup>, De Clerck, O.<sup>1</sup> & Matthijnssens, J.<sup>3</sup>

<sup>1</sup>Phycology Research Group, Department of Biology, Ghent University, Ghent, Belgium <sup>2</sup>Laboratory of Microbiology, Department Biochemistry and Microbiology, Ghent University, Ghent, Belgium <sup>3</sup>Laboratory of Viral Metagenomics, KU Leuven, Leuven, Belgium \*luna.vanderloos@ugent.be

Viral infections could have a substantial impact on the ecology and aquaculture of seaweeds, but surprisingly little is known about virus diversity in macroalgal hosts. Using metagenomic sequencing, we characterized viral communities associated with healthy and bleached specimens of the commercially important green seaweed *Ulva*. We identified 20 putative new and divergent viruses, of which the majority belonged to the CRESS viruses (single-stranded (ss)DNA genomes), *Durnavirales* (double-stranded (ds)RNA), *Picornavirales* (ssRNA). Other newly identified RNA viruses were related to the *Ghabrivirales*, the *Mitoviridae*, and the *Tombusviridae*. Bleached *Ulva* samples contained particularly high viral read numbers. While the CRESS DNA viruses and picorna-like viruses were (nearly) absent from the healthy *Ulva* samples, they were very abundant in the bleached specimens. Therefore, bleaching in *Ulva* could be caused by one or a combination of the identified viruses, but may also be the result of another causative agent or abiotic stress, with the viruses simply proliferating in already unhealthy seaweed tissue. This study highlights how little we know about the diversity and ecology of seaweed viruses, especially in relation to health and diseases of the algal host, and emphasizes the need to better characterize the algal virosphere.

#### Immune resistance in the red alga *Dasysiphonia japonica* against the oomycete pathogen *Olpidiopsis heterosiphoniae*

#### Wen, X.\* & Kim, G.H.

Department of Biological Sciences, Kongju National University, Korea \*wenxianying0310@gmail.com

Oomycete pathogens cause diseases in red algae than can cause enormous economic losses. The ability to recognize and respond to the presence of pathogens is an essential strategy to survive in marine environments, but our understanding of effective defense mechanisms in red algae is limited. The red alga *Dasysiphonia japonica* is infected by *Olpidiopsis heterosiphoniae*, an oomycete, but the algae can coexist with the host for more than 5 years in culture, with the highest infection rate when first exposed to the pathogen (4 days) and infection decreasing over time, but still maintained. Transcriptomic analysis was performed at 4- and 30-days after infection to investigate host's disease defense mechanisms that survive and are not dissipated from infection of the entire thallus by the pathogen. In total, 19,475 upregulated and 907 down-regulated genes were identified from all infection groups. Some genes increased in expression 4-days post-infection and decreased 30-days post-infection (e.g., cellulose synthases, LRR containing domain proteins, R proteins), while some genes maintained high levels, over controls, at both 4- and 30-days (e.g., glycoproteins, casein kinase I, oxidoreductases). Our results provide molecular evidence for early pathogen defense and long-term pathogen defense mechanisms in *D. japonica*. We expect that our transcriptome results will serve as a starting point for understanding various disease defense mechanisms in red algae, which are still a mystery.

## SYMPOSIUM 05 "New insights into the mechanisms and regulation of life cycles in algae"

### Role of the genome-wide distribution of the epigenetic marks H3K27ME3 and H3K4ME3 in the microalgae Ostreococcus tauri

Arvanitidou, C.<sup>1\*</sup>, García-González, M.<sup>2</sup> & Romero-Campero, F.J.<sup>2</sup>

<sup>1</sup>Institute of Plant Biochemistry and Photosynthesis, University of Seville - Consejo Superior de Investigaciones Científicas, Seville, Spain

<sup>2</sup>Department of Computer Science and Artificial Intelligence, University of Seville, Seville, Spain \*carvanitidou@us.es

Epigenetics is one of the major adaptative regulatory mechanisms controlling gene expression in eukaryotes with histone post-translational modifications affecting chromatin accessibility and gene expression. Nevertheless, little relevant genome-wide information is available for chlorophyta. In this study, a protocol to generate ChIP-seq for the epigenetic marks H3K27me3 and H3K4me3 was developed for the marine chlorophyta *Ostreococcus tauri*. Our analysis characterized the genomic regions occupied by these two antagonist marks revealing an occupation of the entire gene body by H3K27me3 and a specific localization of H3K4me3 at the transcriptional start site. H3K4me3 was associated with gene activation whereas H3K27me3 with gene silencing. The distribution of both marks was studied under Long Day conditions (16h light: 8h darkness) at ZT8 and ZT16 at 20°C. No

differences were observed, indicating that these epigenetic marks do not regulate circadian rhythms in *Ostreococcus*. Nevertheless, changes in epigenetic mark distribution were observed when the culture was submitted to temperatures higher and lower than the optimal temperature of 20°C. The temperatures tested were 10°C, 14°C, 24°C and 26°C. Our results indicate that H3K27me3 differentially marked genes are involved in cell cycle progression and fatty acid profiles remodelling. Whereas H3K4me3 differentially marked genes codify for components of the systems for misfolded proteins quality control. RNA-seq data were generated from the same conditions and integrated with the corresponding ChIP-seq data in order to determine how changes in H3K27me3 and H3K4me3 affect gene expression as a response to temperature stress.

#### Formation and dynamics of the guanine crystals regarding the light adaptation of dinoflagellate *Amphidinium carterae*

Bokov, M.<sup>1</sup>\*, Hodoš, M.<sup>1</sup>, Bura, R.<sup>1</sup>, Pilátová, J.<sup>1,2</sup> & Mojzeš, P.<sup>1</sup>

<sup>1</sup>Institute of Physics, Faculty of Mathematics and Physics, Charles University, Prague, Czech Republic <sup>2</sup>Department of Experimental Plant Biology, Faculty of Science, Charles University, Prague, Czech Republic \*maxibokov2@gmail.com

Biogenic guanine crystals are essential for light manipulation in animals and photosynthetic microalgae. These crystals, with their high refractive index and plate-like arrangement, serve various functions such as scattering, reflection, and image formation. Interestingly, similar crystalline inclusions have been found in unrelated photosynthetic microalgae. In *Amphidinium carterae*, guanine crystals act as long-term nitrogen stores and potentially play a role in light manipulation.

Using confocal Raman microscopy, we studied guanine crystals in *A. carterae* cells under different light and nutritional conditions. Crystal formation typically occurs at the cell periphery, between the cell wall and plastids, regardless of light intensity. Even under prolonged darkness, nitrogen supplementation triggers guanine crystal formation at the periphery. However, the crystal position can change depending on light stress.

Under conditions of low light intensity and continuous inorganic nitrogen supply, cells adapt by increasing plastid abundance and depositing guanine crystals closer to the cell center. This positioning enhances photosynthetic efficiency by redirecting photons to the plastids. Conversely, under high light intensity, extensive guanine crystal layers form at the cell periphery, providing shielding to the plastids against excessive illumination. This suggests a regulatory role of guanine crystals in photosynthetic efficiency and protection against photodamage in *A. carterae*.

In summary, biogenic guanine crystals are crucial for light manipulation and nitrogen storage in various organisms. In *A. carterae*, these crystals contribute to regulating photosynthetic efficiency and safeguarding plastids under different light conditions

### Biological and sustainable methods to control biofilms in the ornamental fountains of the Alhambra and the Generalife

Bolívar, F.<sup>1\*</sup>, Calvo, I.<sup>1</sup>, Cuberos, L.M<sup>1</sup>, Fillet, S.<sup>2</sup>, Pérez, E.<sup>1</sup>, Bolívar, M<sup>1</sup>, Ballesteros, J.C.<sup>1</sup>, Poyatos, F.<sup>1</sup> & Romero, J<sup>1</sup>.

<sup>&</sup>lt;sup>1</sup>Ligalismo Laboratory. University of Granada. Avda. Andalucía s/n, 18071, Spain. <sup>2</sup>Sanbiotec Laboratory. Plaza Rafael Alberti, 7, 18630, Granada, Spain. \*fbolivar@ugr.es

The monumental complex of the Alhambra and the Generalife, due to its historical and artistic importance, requires multidisciplinary projects that join the capabilities of professionals from various fields to find solutions to microalgae biodeterioration in its ornamental fountains.

Traditionally, attempts have been made to prevent the proliferation of algal biofilms with physical and chemical solutions, which usually cause deterioration of stone materials and are often insufficient to inhibit their appearance and development.

Our research group HUM-1031 LIGALISMO (Arts & Sciences) through the research project "Synergistic Art-Science-Technology Methods for the Conservation-Restoration of the Alhambra and other cultural assets" (VIRARTE II), has found the presence of halos of inhibition of algal growth in the mats present in the fountains that indicate that there may be microorganisms capable of hindering the growth of microalgae. Several samplings have been carried out in these areas, identifying by genetic sequencing the bacterium *Stenophonomas maltophilia* and the fungus *Microdochium bolleyi*, as possible responsible for the inhibition of algal growth.

These results open a new avenue of research for the treatment of biodeterioration of historical fountains using biological algaecides, which are more respectful than classical biocides of the environment and the integrity of the original materials.

#### Photosynthetic performances and photoprotection strategies in life cycle stages of the coccolithophore *Calcidiscus leptoporus*

Chaux, F.\* & Godrijan, J.

Ruđer Bošković Institute Zagreb, Croatia \*fchauxj@irb.hr

Coccolithophores are an important group of calcifying unicellular algae that play a significant role in the global carbon cycle, oceanic ecology, and climate regulation. In contrast with most eukaryotes, they thrive through a haplo-diplontic life cycle: both haploids and diploids can undergo rounds of mitosis. Moreover, within a given species, haploid and diploid cells are generally enclosed in distinct calcareous plates (holo- and heterococcoliths, respectively) and it has been proposed that they display different physiological features to accommodate distinct ecological niches of the ocean.

In this work, we compare physiological differences of haploid and diploid stages of *Calcidiscus leptoporus*, represented by strains from various locations around the globe. We investigated their (i) growth rates in a variety of light, temperature and nutrient conditions and (ii) photosynthetic electron transfers *in vivo* using room-temperature chlorophyll fluorescence. Since the latter method was hardly used before in coccolithophores, we introduce settings for pulse-amplitude modulated measurements, accounting for peculiarities such as the presence of holo- or heterococcoliths and cell densities lower than in model organisms (green algae). Differences in the electron flow saturation curves suggest distinct adaptations to their niche illumination regime. To better understand the distinct strategies in light harvesting optimization, we further recorded the functional antenna size of photosystem II and assessed the components of non-photochemical quenching.

We discuss the relation between observed growth rates, photosynthetic performances, and photoprotection strategies with the original cell locations and their life cycle stages to justify ploidy-dependent physiological adaptations to distinct niches in *C. leptoporus*.

### Developmental signaling during spore division and morphological differentiation in the red algae *Bostrychia moritziana*

Choi, Y.J.\*, Park, H., Shim, E. & Kim, G.H.

Department of Biology, Kongju National University, Gongju, Korea \* mary001116@smail.kongju.ac.kr

The early division of spores is a critical time for determining the final success of an individual, yet little is known about the signals that regulate these early stages in red algae. In this study, we used carpospores of the florideophycean red algae, Bostrychia moritziana, to investigate the role of the cytoskeleton and reactive oxygen species (ROS) signaling during early spore development. The process of spore development begins with an asymmetrical cell division, in which a large cell that will develop into a main axis and a smaller cell that will develop into a rhizoid. Treatment of the released spores with a microtubule inhibitor, oryzalin, resulted in symmetrical division without differentiation of rhizoidal cells, as well as irregular nuclear division and cytoplasmic. Fluorescent labeling of ROS with DCFH-DA showed a large amount of ROS in the cell division plane. When released spores were treated with the NADPH oxidase inhibitor, DPI, for 15 minutes, the initial growth of the spores was delayed compared to controls. When spores were treated with caffeine, a calcium channel inhibitor, spores developed into abnormal for with short main axis and significantly longer rhizoidal part. The spores whose initial growth was disrupted with the inhibitor developed significantly smaller sizes and fewer branches than the control group for more than 4 weeks. These results show that microtubule-mediated cell division and ROS signaling play an important role in early development and that perturbation of these signaling have long-lasting effects on morphogenesis of *B. moritziana*.

#### **Basal Cell Fate Determination in Ectocarpus**

Godfroy, O.<sup>1</sup>\*, Zheng, M.<sup>2</sup>, Uji, T.<sup>1</sup>, Colin, S.<sup>2</sup>, Ronchi, P.<sup>3</sup>, Nagasato, C.<sup>4</sup>, Motomura, T.<sup>4</sup>, Cock, J.M.<sup>1</sup> & Coelho, S.M.<sup>2</sup>

 <sup>1</sup>UMR 8227 CNRS-Sorbonne University, Integrative Biology of Marine Models, Station Biologique de Roscoff, France
 <sup>2</sup>Max Planck Institute for Developmental Biology, Tübingen, Germany
 <sup>3</sup>Electron Microscopy Core Facility, European Molecular Biology Laboratory, Heidelberg, Germany
 <sup>4</sup>Muroran Marine Station, Hokkaido University, Japan
 \*godfroy@sb-roscoff.fr

In the model brown alga *Ectocarpus* sp.7, the independent gametophytic (GA) and sporophytic (SP) generations deploy a distinct developmental program autonomously from an initial cell. Both generations possess a simple thallus organization composed of few cell-types that can be grouped into 1) a basal body part, allowing attachment to the substratum (rhizoids in GA and rhizoids and prostrate filaments in SP) and 2) an erected body part that bears the reproductive structures (filaments in GA and upright filaments in SP).

We identified mutants affected in two different genes, *DISTAG* and *BASELESS*, which lost basal structures in both generations. Several cellular abnormalities are observed in the initial cell including disruption of the germination pattern, modification of cytoskeleton organisation, nucleus mispositioning and Golgi apparatus fragmentation. Altogether, those phenotypes emphasize the crucial role of the first cell division in the establishment of major developmental axes and cell fates and the importance of these genes for pattern development in both the gametophyte and sporophyte.

*DISTAG* and *BASELESS* encode, respectively, a Tubulin-binding co-factor C d1 (TBCCd1) protein and a Ca<sup>2+</sup>-sensitive type B" regulatory subunit of protein phosphatase 2A (PP2A), highlighting the role of the cytoskeleton and Ca<sup>2+</sup> signalling in the initial cell division. Orthologues of both proteins regulate internal cell organization in plants and animals demonstrating the deep evolutionary conservation of those intra-cellular processes.

#### Changes of nuclear DNA content estimates during the life cycle stages of sugar kelp, *Saccharina latissima*

Goecke, F.<sup>1</sup>\*, Gómez Garreta, A.<sup>2</sup>, Martín Martín, R.<sup>2,3</sup>, Rull Lluch, J.<sup>2</sup>, Skjermo, J.<sup>4</sup>, & Ergon, Å.<sup>1</sup>

<sup>1</sup>Department of Plant Sciences, Faculty of Biosciences, Norwegian University of Life Sciences, Ås, Norway <sup>2</sup>Laboratori de Botànica, Facultat de Farmàcia i Ciències de l'Alimentació; Institut de Recerca de la Biodiversitat (IRBio) & Centre de Documentació de Biodiversitat Vegetal (CeDocBiV), Universitat de Barcelona, Barcelona, Spain

<sup>3</sup>Département de biologie, chimie et géographie, Université du Québec à Rimouski (UQAR) & Merinov, Canada

<sup>4</sup>SINTEF Ocean, Trondheim, Norway

\*franz.goecke@nmbu.no

Ploidy variants can be utilized to increase yield, introduce sterility, and modify other specific traits with an economic impact. DAPI microfluorometric analysis, flow cytometry, and chromosome counting are three of the most common methods to determine ploidy in plants. Despite the economic importance of Saccharina species, their nuclear DNA content of different cell types and life stages remain unclear. The present research was initiated to determine ploidy and nuclear DNA content at different life cycle stages of Saccharina latissima. Nuclear DNA content in embryonic and mature sporophytes, released and unreleased zoospores, female, and male gametophytes from Sør-Trøndelag county in Norway were estimated by image analysis using the DNA-localizing fluorochrome DAPI and chicken's red blood cells as a standard. DNA content of a total of 6905 DAPI-stained nuclei was estimated. This is the first C-value study which covered the whole life cycle of a Laminarian kelp, with the only exception of gametes. We observed considerable variation of DNA content estimates during the life cycle stages of sugar kelp, and within adult sporophytes. Zoospores presented the lowest level of DNA content (1C), but single spore gametophyte cultures presented higher average DNA content, suggesting the presence of polyteny. Embryonic sporophytes and older sporophytes' meristoderm cells presented 2C as expected. Mature sporophytes showed large intra-plant variation with DNA content values ranging from 2-16C. DAPI microfluorometric analysis can be used for DNA content determination in kelp but assumptions about ploidy level must be supported by chromosome counting.

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#### Exploring the role of epigenetic mechanisms in the salinity stress response of the model diatom *Phaeodactylum tricornutum*

Groisillier, A.\*, Yang, F. & Tirichine, L.

Nantes Université, CNRS, US2B, UMR 6286, F-44000 Nantes, France \* agnes.groisillier@univ-nantes.fr

The salinity gradients in estuarine and coastal areas are highly dynamic, with hourly changes occurring during tidal cycles and monthly changes during seasonal cycles. These fluctuations are largely attributed to the effects of climate change and human activities and are expected to persist in the future. Alterations in salinity levels have notable effects on marine life, particularly on phytoplankton, which occupy a critical position at the base of the food chain. Diatoms, among the most successful phytoplankton groups, are significant contributors to both photosynthetic carbon fixation and primary productivity, accounting for approximately 20% and 40%, respectively. Thus salinity fluctuations play a critical role in shaping the ecological functions of diatoms and their impact on marine ecosystems. In this context, organism's responses to stress involve a rapid switch from the regular developmental program to the stress program known to involve an epigenetic mediated regulation of the response. Therefore, it is critical to include the study of epigenetic mechanisms to comprehensively understand diatom responses to salinity stress. The focus of our study was to examine how salinity changes impact the physiology of cells using the model diatom, *Phaeodactylum tricornutum*. Additionally, we aimed to explore the role of epigenetic mechanisms, including DNA methylation and Post Translational Modifications of histones, in the cellular response to changes in salinity levels.

#### Development of sexual type PCR markers for Ulva spp.

Habchi, K.<sup>1</sup>\*, Nehr, Z.<sup>1</sup>, Lipinska, A.P.<sup>2</sup>, Fort, A.<sup>3</sup>, Potin, D.<sup>1</sup>, Le Panse, S.<sup>4</sup>, Gachenot, M.<sup>5</sup>, Sulpice, R.<sup>3</sup> & Potin, P.<sup>1</sup>

<sup>1</sup>Integrated Biology of Marine Models UMR8227, Station Biologique de Roscoff, France <sup>2</sup>Department of Algal Development and evolution, Max Planck Institute for Biology, Tubingen, Germany <sup>3</sup>Plant Systems Biology Lab, Ryan institute & MaREI Centre for Marine, Climate and Energy, School of Natural Sciences, National University of Ireland - Galway, Galway, Ireland <sup>4</sup>Plateforme d'Imagerie Merimage, FR2424, Sorbonne Université, CNRS, Station Biologique de Roscoff, Roscoff, France <sup>5</sup>Plateforme de Cytométrie RECYF FR2424, Sorbonne Université, CNRS, Station Biologique de Roscoff,

France

\*rahmakawtherhabchi@gmail.com

Green macroalgae are widely spread across the coastline of all the continents. Among them, some *Ulva* species are world renowned for green tides and others grab the interest of the aquaculture production due to fast growth, high nutritive value, bioactive products and bioremediation potential. *Ulva spp.* have an haplo-diplobiontic sexual life cycle with alternance between sexual and asexual stage. However, data on life cycle dynamics have been limited owing to the difficulties in determining the ploidy or sex using morphological characters of thalli as well as the lack of reliable molecular markers. We used *Ulva* strains from the 3 main species occurring in Brittany, France, to identify the triggers of reproductive development. To identify the main factors involved in gametogenesis induction, we subjected the seaweeds to several conditions such as thermal shock, various salinities, full moon and various nutrient status, which can stress the algae and activate its gametogenesis. Then we determined morphologically the mating type of the obtained gametes on which we validated the mating type markers. To generate these markers, we used Illumina sequences obtained from a strain collection and we searched for homologous sequences to *Ulva partita* mating type locus. For the field of algal cultivation, this work will provide tools to isolate and produce axenic strains, to efficiently determine mating type and ploidy and to make controlled crosses and genetic breeding of *Ulva spp*.

### Molecular mechanisms of spore movement and substrate attachment of red alga *Bangia* sp.

Kim, S.Y.\*, Shim, E. & Kim, G.H.

Department of biological sciences, Kongju National University, Gongju, Korea \*replex1@smail.kongju.ac.kr

Locomotion in free-living cells is an active response of an organism to its environment and involves a process of perception of stimuli, signaling for cellular responses, and subsequent systematic changes in the cytoskeleton. Red algae are considered non-motile as they have a very poor gene repertoire of cytoskeletal molecules. Bangia sp. is cultivated in East Asia due to its commercial value. Spores of Bangia sp. have amoeboid motility for a significant period of time prior to attachment to the substrate, so understanding why and how long they move, and the mechanism of motility is essential to developing successful seed culture techniques. Changes in actin distribution occurred when spores attached to the substrate, and the attachment was inhibited when spores were treated with cytochalasin D. Spore motility of Bangia sp. was most affected by temperature. At temperatures below 10°C, spores lost motility and attached to the bottom within a day, while at temperatures above 25°C, they remained motile for more than three days. Changes in movement patterns were observed upon treatment with the Phosphoinositide 3-kinase (PI3K) inhibitors Wortmannin, suggesting that polymerization of actin during spore movement is mediated through the PI3K-Protein kinase B (Akt) pathway. Actin related protein 2/3 complex (ARP2/3) genes involved in the branching of actin microfilaments were identified and their expression was analyzed at different stages of spore movement. Spore motility in red algae, will provide important clues to the origin of cellular motility, and may aid in mediating the attachment of *Bangia* sp. to farm infrastructure.

#### Development of gene probes to track sexual reproduction events in diatoms

Rijsdijk, N.<sup>1,2,3</sup>\*, Bilcke, G.<sup>1,2,3</sup>, Chaerle, P.<sup>1</sup>, Vandepoele, K.<sup>2,3</sup> & Vyverman, W.<sup>1</sup>

<sup>1</sup>Labotory of Protistology and Aquatic Ecology, Ghent University, Belgium <sup>2</sup>Department of Plant Biotechnology and Bioinformatics, Ghent University, Belgium <sup>3</sup>Vlaams Instituut voor Biotechnologie, Center for Plant Systems Biology, Ghent, Belgium

\*Nadine.Rijsdijk@Ugent.be

Sexual reproduction is an essential aspect in the life cycle of most diatoms, the most speciose group of microalgae. Diatom cell size decreases during vegetative growth by consecutive cell divisions. Typically, cell size is restored through the development of an expanding zygote called the auxospore. Not only does sexual reproduction avoid critical miniaturization, it also maintains genetic diversity and is thought to play an essential role in adaptation to changing environments. However, for various reasons, sexual reproduction has rarely been observed in nature.

Here, we aim to develop gene probes that can be used to track sexual events in natural diatom populations. Comparative transcriptomics analysis of bulk RNA-sequencing data of several centric and pennate diatoms showed orthologous genes solely upregulated during sexual reproduction that can be used as sex marker genes (Bilcke *et al.*, unpubl., Audoor *et al.*, unpubl.). Following optimization of experimental protocols, we profiled sex transcriptomes of additional species to further constrain the number of candidate sex marker genes. Next, we conducted phylogenetic analysis to assess sequence conservation of these genes and their taxonomic applicability as sex marker probes. To assess the

feasibility of using these genes as sex markers as well as their sensitivity, we performed qPCR of selected genes in the pennate diatom *Cylindrotheca closterium*. The development of sex marker probes will be an important step for understanding the phenology of sexual reproduction in natural diatom populations.

# Effects of temperature and irradiance on the vegetative propagation processes in gametophytes of *Asparagopsis armata* (Florideophyceae, Rhodophyta) mediated by hook-like structures

Sainz-Villegas, S.\*, Sánchez-Astráin, B., Puente, A. & Juanes, J.A.

IHCantabria - Instituto de Hidráulica Ambiental de la Universidad de Cantabria, Santander, 39011, Spain \*samuel.sainz@unican.es

The red seaweed Asparagopsis armata is widely distributed in the European coasts of the North Atlantic, where it is considered an introduced and invasive species. However, it has been recently shown that this species produces certain metabolites with the capacity to reduce methane production of ruminants when added to those animals' diet. Those findings have increased the interest and demand of this species globally. Here, we examined the effects of temperature and irradiance on the vegetative propagation process of gametophytes in a laboratory experiment. We considered vegetative propagation due to its implications in the invasive process (as it is considered the main source of recruitment) and to its importance in seaweed aquaculture. The process was characterized through the survival rates of hookshaped specialized structures and the production and growth of new plantlets from hooks of 13cm in length. Results revealed how vegetative propagation for this species was conditioned by the low survival rates of hooks once detached from the main thallus. In our experiment, survival probabilities after 30 of culture were always below 50%. Comparisons among environmental conditions showed lower survival rates under increasing levels of temperature and decreasing levels of irradiance. In fact, mass mortality was detected at temperatures over 18°C, where hook-shaped fragments did not survive more than 10 days. Nevertheless, patterns of appearance and growth of plantlets at different temperatures and irradiances were not clear. In the light of climate change, understanding these reproduction patterns is necessary in order to adopt better management actions in the future.

#### Whether and how do polyphenols affect epiphytic diatom biomass?

Strugała, M.\* & Messyasz, B.

Department of Hydrobiology, Faculty of Biology, Adam Mickiewicz University in Poznan, Uniwersytetu Poznanskiego 6, 61-614 Poznan, Poland

Polyphenols are organic chemical compounds that occur naturally in plants. They are, among others, produced by macroalgae *Cladophora glomerata* (L.) Kützing, filamentous green algae that inhabit aquatic ecosystems that are strongly eutrophic. *Cladophora* is a species often colonized by periphyton organisms that settle on the surface of the objects submerged in the water. Colonization by diatoms of the single strand of *Cladophora* can occur in various ways, and it is influenced by the architecture and surface roughness (e.g. calcium carbonate crystals) of the substrate, but also by polyphenols secreted by this green macroalga. Such a relationship may affect the formation of dominant taxa in the periphyton community composition, as some epiphytic diatoms will disappear due to the polyphenols secretion by *Cladophora*. The aim of the study was to determine how individual species of diatoms react to the

increasing concentration of polyphenols in water and which of them colonize and multiply the fastest on *Cladophora* in the event of an increased amount of this chemical compound.

The conducted research showed that individual species of diatoms inhabiting the green alga reacted differently to the content of polyphenols in the microhabitat. Three groups were defined - neutral diatoms, positively reacting diatoms and negatively reacting diatoms, from which dominants were selected, which will participate in the next experimental research on the use of diatoms inhabiting *Cladophora*.

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### Phenotypic plasticity in early development of Dictyota dichotoma

Zakka, E.E.<sup>1\*</sup>, Bogaert, K.A.<sup>2</sup> & De Clerck, O.<sup>2</sup>

<sup>1,2</sup>Phycology Research Group, Ghent University, Belgium \*eliane.zakka@ugent.be

*Dictyota dichotoma* is an oogamous brown seaweed. Its zygotes typically undergo an asymmetric division resulting in an apical and a basal cell. The latter will give rise to a rhizoid that attaches the zygote.

Here we report an alternative developmental pathway whereby the initial zygote undergoes a symmetric division and rhizoid formation is inhibited. *Dictyota* therefore undergoes phenotypic plasticity with the ability to modulate between symmetric and asymmetric cell division. This phenotypic plasticity manifests itself under high densities of developing zygotes or in the presence of vegetative tissue.

We hypothesize the existence of a metabolite, produced by the vegetative tissue, that interfere with asymmetrical cell division and rhizoid formation. We report on a series of experiments aimed at narrowing down the identification of the metabolite. The metabolite of interest was shown to withstand UV degradation, can be stored at -80 °C and is volatile. SPME-GC-MS will serve in identifying the molecular nature of the volatile rhizoid-inhibiting metabolite.

Furthermore, related species of *Dictyota* also have an inhibitory effect on *Dictyota dichotoma* zygotes, whereas more distantly related representatives of the Dictyotales showed almost no inhibitory effect.

### SYMPOSIUM 06 "Algal cell biology and ultrastructure"

### Global transcriptome of short- and long-term hyposaline acclimation in a marine diatom: links to the non-coding genome

Charton, F.<sup>1\*</sup>, Debit, A.<sup>1</sup>, Bowler, C.<sup>1</sup> & Cruz de Carvalho, H.<sup>1,2</sup>

<sup>1</sup>Institut de Biologie de l'ENS (IBENS), Département de Biologie, École normale supérieure, CNRS, INSERM, Université PSL, 75005 Paris, France

<sup>2</sup>Université Paris Est-Créteil (UPEC), Faculté des Sciences et Technologie, 61, avenue du Général de Gaulle 94000 Créteil, France

\*charton@bio.ens.psl.eu

Over the last decade, the interest in the study of the non-coding genome and more particularly of long non-coding RNAs (lncRNAs) has been growing exponentially. While lncRNAs are increasingly well characterized in multicellular organisms, notably in the regulation of developmental processes, stress

response or cell differentiation, they remain poorly characterized in other living organisms, namely unicellular microalgae. Previous works have demonstrated the presence of lncRNAs in diatoms, in particular *Phaeodactylum tricornutum*, where their expression is specifically correlated with environmental fluctuations (nutrients, light,  $pCO_2...$ ).

Recently, we have shown that in transcriptomic datasets associated with pleomorphic changes in *P. tricornutum*, lncRNAs cluster according to a morphotype, indicating a morphotype-specific "transcriptomic signature". However, we also provide evidence suggesting that the hyposaline culture conditions used to generate one of the morphotypes likely overshadowed the morphotype response. We therefore decided to investigate further this aspect by focusing on the short- and long-term acclimation to and from hyposaline culture conditions in this marine diatom.

A global study to understand how *P. tricornutum* responds to a drastic change in the salinity of its environment in the first few days and, in general, how cells acclimate after longer periods under hyposaline conditions was undertaken. I will present our most recent results both in terms of the cellular responses as well as the underlying transcriptomic responses (coding and noncoding).

## PhaeoEpiView: An epigenome browser of the newly assembled genome of the model diatom *Phaeodactylum tricornutum*

Chaumier, T.<sup>1¥\*</sup>, Wu, Y.<sup>1¥</sup>, Manirakiza, E.<sup>1</sup>, Veluchamy, A.<sup>2</sup> & Tirichine, L.<sup>1</sup>

<sup>¥</sup> Equal contribution

<sup>1</sup>Nantes Université, CNRS, US2B, UMR 6286, F-44000 Nantes, France

<sup>2</sup>St Jude Children's Research Hospital, Memphis, TN, US

\*timothee.chaumier@univ-nantes.fr

Recent advances in DNA sequencing technologies particularly long-read sequencing, greatly improved genomes assembly. However, this has created discrepancies between published annotations and epigenome tracks, which have not been updated to keep pace with the new assemblies.

Here, we used the latest improved telomere-to-telomere assembly of the model pennate diatom *Phaeodactylum tricornutum* to lift over the gene models from Phatr3, a previously annotated reference genome. We used the lifted genes annotation and newly published transposable elements to map the epigenome landscape, namely DNA methylation and posttranslational modifications of histones. This provides the community with PhaeoEpiView, a browser that allows the visualization of epigenome data and transcripts on an updated and contiguous reference genome, to better understand the biological significance of the mapped data. We updated previously published histone marks with a more accurate peak calling using mono instead of poly(clonal) antibodies and deeper sequencing.

PhaeoEpiView (https://PhaeoEpiView.univ-nantes.fr) will be continuously updated with the newly published epigenomic data, making it the largest and most comprehensive epigenome browser of any stramenopile. Particularly, to better understand the population molecular and phenotypical diversity of *P. tricornutum* populations, as well as clade-specific features, we plan to integrate genomic and epigenetic data of other *P. tricornutum* accessions. We believe that PhaeoEpiView will become a widely and frequently utilized tool in the forthcoming age of molecular environmental studies, where epigenetics plays a crucial role.

# Discovery of red algal carbohydrate sulfotransferases and their implication in cell wall biosynthesis

Chevenier, A.\*, Michel, G. & Ficko-Blean, E.

CNRS, Sorbonne Université, Laboratory of Integrative Biology of Marine Models, Station Biologique de Roscoff, France \*antonin.chevenier@sb-roscoff.fr

Red algae and land plants both belong to the Archaeplastida and share some similarities in the composition of their cell wall (CW), like the presence of cellulose. Nonetheless, the major constituents in some red algal CW matrices are sulfated polysaccharides (SPs) which are absent in land plants. Interestingly, metazoans possess SPs in their extracellular matrices (ECMs) as well.

Some species of red algae contain sulfated galactans called carrageenans. During carrageenan biosynthesis, sulfate esters are thought to be enzymatically added by regiospecific carbohydrate-sulfotransferases (CSTs). Recently, the sequencing of the genome of the red algal carrageenophyte *Chondrus crispus* predicted several CST genes. These sequences are homologues to animal CSTs involved in the biosynthesis of sulfated glycosaminoglycans which suggests similarities in the mechanism of polysaccharide sulfurylation between algae and metazoans. This has resulted in the hypothesis that the *Chondrus* CSTs are involved in carrageenan biosynthesis.

Studying CST biochemistry is essential to understanding CW biosynthesis in red algae. Furthermore, resolving the mechanisms of carrageenan synthesis would greatly improve our understanding on the evolution of ECM in eukaryotes. Biochemical studies on recombinant red algal biosynthetic enzymes such as CSTs are challenging, mainly due to protein production problems. This project thus attempts to undertake the first biochemical study into the discovery and characterization of red algal sulfotransferases with the use of different expression systems for heterologous production of *Chondrus* CSTs.

#### Activity-based protein profiling (ABPP) of the green microalga Lobosphaera incisa for identification of functional serine hydrolases

Dalmia, A.\*,<sup>#</sup>, Kumar Dolui, A.<sup>#</sup>, Htet, H.Y. & Khozin-Goldberg, I.

<sup>#</sup>equal contribution

Microalgal Biotechnology Laboratory, The French Associates Institute for Agriculture and Biotechnology of Drylands, Jacob Blaustein Institutes for Desert Research, Ben-Gurion University of the Negev, Midreshet Ben-Gurion, 8499000, Israel \*ayushidalmia@yahoo.co.in

Lobosphaera incisa is a green microalga capable of accumulating large amounts of the biologically important omega 6 long-chain polyunsaturated fatty acid (LC-PUFA), arachidonic acid (ARA, 20:4n-6). *L. incisa* is one of the few known species accumulating LC-PUFA in triacylglycerols (TAG). This feature makes it a biotechnologically potent strain. Nitrogen (N) starvation leads to ARA-rich TAG accumulation and growth arrest. After nutrient replenishment, the acyl groups of TAG are used as an energy source to support growth recovery and restoration of plastidial membrane lipids. The breakdown of TAG occurs rapidly, indicating the presence of robust lipases. In the present study, we used activity-based protein profiling (ABPP) to identify functional serine hydrolases (SH), including lipases, in the *L. incisa* cells after 7 days of N starvation and 24 hours after nutrient resupply. Global proteome analysis revealed the twenty six differentially expressed proteins, involved in lipid metabolism, between nitrogen starvation and recovery. Gel-based ABPP using a TAMRA-SH probe, with and without SH inhibitors, revealed functionally active SHs during N starvation and recovery. The identified SHs belong to six groups namely, lipases, including class 3 lipases, GSDL lipases and phospholipases, serine proteases, thioesterases, acyltransferases and metabolic SHs. This study facilitates the understanding of the role played by SHs in lipid metabolism of *L. incisa*. We surmise that these finding of this work will help to

put together the pieces of the puzzle of the previously observed lipophagy-like process in *L. incisa*. The results of this study may have potential biotechnological applications.

### Building Interactomes in the Cyanobacterium *Synechococcus elongatus* PCC 7942 Using Large-scale Affinity Purification Mass Spectrometry

Dowson, M.\*<sup>1</sup>, Perrin, A.<sup>1</sup>, Zhao, G.<sup>2</sup>, Dowle, A.<sup>1</sup> & Mackinder, L.<sup>1</sup>

<sup>1</sup>Department of Biology, University of York, Wentworth Way, York, YO10 5DD <sup>2</sup>College of Life Science, Shandong Normal University, Jinan, China, 250014 \*matt.dowson@york.ac.uk

Photosynthetic prokaryotes utilise unique cellular biology to contribute towards their key role in global carbon cycling and aquatic ecosystems. An example of this in cyanobacteria is their use of a  $CO_2$  concentrating mechanism to improve the efficiency of photosynthesis using protein microcompartments called carboxysomes. These remarkable processes mean cyanobacteria are also proving to be an emerging player in a range of biotechnological applications.

Despite their biological importance, cyanobacterial genomes are relatively poorly annotated. To develop our understanding of these cellular processes, we used high-throughput techniques to generate a library of *S. elongatus* PCC 7942. Each line intorduces a scarless insertion of a C-terminal mNeonGreen affinity tag into each target protein with a view to eventually have whole-proteome coverage.

A subset of these tagged lines were used to optimise and perform affinity purification mass spectroscopy (AP-MS), allowing for discovery of high confidence protein interactors for a broad range of tagged protein baits. Analysis of the label-free proteomic data provides insight into some of the most significant protein-protein interactions, resulting in a better understanding of the complexes involved in key cyanobacterial cellular functions including photosynthesis and CO<sub>2</sub> concentration as well as providing insight into potential novel interactions in these processes for further study. From this we have started to build a whole cell protein interactome to show the connectedness of the cyanobacterial proteome.

# Cell repair in *Griffithsia monilis* mediated by ROS and calcium signaling

Hong, C.Y.\* & Kim, G.H.

Algae Laboratory, Department of Biological Sciences, Kongju National University, Republic of Korea nicegandy@smail.kongju.ac.kr

The process by which its cellular membranes and internal structures are repaired to restore function is called "cell repair". In plants, little is known about how cells repair when both the cell wall and cell membrane are damaged. Using the red alga *Griffithsia monilis* as a model, we studied the cell repair process.

When cell was punctured, a large amount of cytoplasm leaked out from the cell. However, immediately after the wound was formed, it was covered with a gelatinous substance plugging the wound, and within an hour, the cell's osmotic pressure was restored and the cell returned to its normal shape. During the repair process, chloroplasts were initially clustered around the wound and then re-distributed evenly on the cell surface over 48 hours, and nuclei around the wound also underwent a relocation process. Experiments using fluorescent probes and inhibitors of the cytoskeleton showed that this process is mediated by microtubules. Using DCFH-DA and Fluo-8 AM staining, we observed high accumulation

of reactive oxygen species (ROS) and  $Ca^{2+}$  at the wound site. Treatment with DPI, an inhibitor of NADPH oxidase (RBOH) that produces intracellular ROS, and ascorbic acid, an antioxidant, prevented cell repair. Expression of *RBOH* genes, which mediate ROS production and *CDTKL* gene, which is involved in  $Ca^{2+}$  signaling were investigated during cell repair.

The results of cell repair using *Griffithsia monilis* are expected to provide useful scientific data for the study of plant cell repair mechanisms that have not been studied much.

# Profiling histone post-translational modifications in three green algae species

Krela, R.<sup>1\*</sup>, Sreekumar, S.<sup>1</sup>, Lochmanová, G.<sup>2</sup>, Bišová, K.<sup>3</sup>, Zdráhal, Z.<sup>2</sup> & Mozgová, I.<sup>1</sup>

<sup>1</sup>Biology Centre, Czech Academy of Sciences, Institute of Plant Molecular Biology, České Budějovice, Czech Republic

<sup>2</sup>Central European Institute of Technology, Masaryk University, Brno, Czech Republic <sup>3</sup>Centre Algatech, Czech Academy of Sciences, Institute of Microbiology, Laboratory of Cell Cycles of Algae, Trebon, Czech Republic \*rafal.krela@umbr.cas.cz

Epigenetic regulation is mediating transcriptional activity in response to environmental changes. Because of complex growth patterns and requirements, understanding the epigenetic machinery and targets is crucial for optimizing the growth of green algae. Mass spectroscopy allows to quantitative examine the presence and combination of histone epigenetic modifications, while ChIP\_ qPCR/-Seq are powerful tools to assess gene targets of these epigenetic marks. We present an approach to quantitatively and qualitatively profiling histone PTMs in three different green algae species (*Chlamydomonas reinhardtii, Scenedesmus quadricauda,* and *Chlorella sorokiniana*).

We applied an unbiased MS to identify and characterize histone H3 variants and histone PTMs in three algae species. Our results show a high diversity of composition and relative quantities of modifications in different species, with Chlamydomonas lacking the facultative heterochromatin mark - H3K27me3.

To describe the gene targets of PTM histone modifications, we adapted ChIP to use in two previously not examined green algae species. We optimized crucial steps such as cell harvesting, crosslinking of histone-DNA complex, fragmentation, co-immunoprecipitation, and the optimization of final ChIP steps: qPCR and libraries preparation for sequencing. We showed the same method with slight changes could be used to examine epigenetic modifications in distinct species, with different cell wall compositions and number of connected cells.

Our straightforward approach allows wider utilization of epigenetic studies using ChIP method in algae research. A new tool to examine epigenetic changes in organisms with dynamic adaptation to environmental changes would lead to the discovery of genes important for the growth and production of ingredients.

# Studying a B12-regulated promoter in *Phaeodactylum* and *Chlamydomonas*

Mendoza-Ochoa, G.I.\*, Zhang, D., Newsad, S.A., Llavero-Pasquina, M., Capp, S. & Smith, A.G.

Department of Plant Sciences, University of Cambridge, UK \*gim23@cam.ac.uk Approximately half of all microalgal species depend on vitamin B12 (cobalamin) as a co-factor for methionine synthase (METH). There is another form of the enzyme, METE, that does not require B12, although it is less efficient in catalysis. Some B12-independent algae, such as Chlamvdomonas reinhardtii and Phaeodactylum tricornutum, encode both METH and METE, enabling them to utilize B12 if it is available. In the presence of B12, the expression of the METE gene is strongly repressed through its promoter, allowing the cell to preferentially employ METH. This regulatory switch has facilitated the use of METE promoters as tools for transgene regulation in Chlamydomonas and Phaeodactylum. Firstly, we investigated the regulatory mechanisms governing the METE promoter (PMETE) in *Phaeodactylum*. We identified a DNA motif within the PMETE that is essential for METE expression and B12 repression, and through yeast one-hybrid assays, we found a transcription factor that binds to the motif. In contrast to the tight repression seen in Phaeodactylum, Chlamydomonas PMETE exhibits leakiness when removed from its native genomic context, such as when used to regulate transgenes. To explore this phenomenon, we generated and analysed a series of transgenic lines containing different elements of the METE gene. Our preliminary analysis revealed the existence of other critical elements, located within the C-terminal end of the coding sequence and its 3' UTR, which are necessary for regulating this gene at levels comparable to the wild-type METE. Overall, our findings provide valuable insights into the mechanisms underlying B12-dependent gene regulation in algae.

## Dissecting the pyrenoid composition of the red alga *Porphyridium purpureum*

Musiał, S.<sup>1,2\*</sup>, Barrett, J.<sup>1</sup>, Blaza, J.<sup>2</sup> & Mackinder, L.<sup>1</sup>

<sup>1</sup>Centre for Novel Agricultural Products (CNAP), Biology Department, University of York, York, UK <sup>2</sup>York Structural Biology Laboratory, Chemistry Department, University of York, York, UK \*Sm2124@york.ac.uk

Rubisco catalyses one of the most important reactions on the planet introducing  $CO_2$  into the photosynthetic Calvin cycle. Many algae enhance their photosynthesis using  $CO_2$  Concentrating Mechanisms (CCMs). One of the elements of this mechanism is a Rubisco condensate called the pyrenoid. The aggregation of Rubisco in one location and increase of  $CO_2$  concentration in its vicinity enhances Rubisco's activity, while the spatial separation of the carboxylation reaction from other components of photosynthesis allows the exclusion of oxygen, preventing an undesirable oxygenation reaction.

Algal pyrenoids display many diverse morphologies and are thought to have evolved multiple times. Despite this, many algal lineages remain understudied in the context of their pyrenoids and CCMs. We aim to bridge that gap in knowledge by studying the structure and function of proteins involved in pyrenoid formation in the red alga *Porphyridium purpureum*. A bioinformatic search revealed a putative intrinsically disordered Rubisco-linker protein which could be involved in biogenesis of the pyrenoid matrix potentially by liquid-liquid phase separation as seen in the model green algal system *Chlamydomonas reinhardtii*. Rubisco co-immunoprecipitation followed by mass spectrometry identified this proposed Rubisco-linker as an abundant interactor along with additional putative pyrenoid components. These candidate pyrenoid proteins are currently in the process of validation via *in vitro* assays and by using fluorescent tagging to confirm their subcellular localisation. These results will then guide the determination of protein structures using cryo-electron microscopy.

We envision that this research will help uncover the general shared principles of pyrenoid assembly - a vital microcompartment for global carbon cycling.

## Molecular and cellular effects of trivalent and hexavalent chromium in *Chlamydomonas reinhardtii*

Normant, V.\*

Université Lorraine, CNRS, LIEC, F-57000 Metz, FRANCE \*Vincent.normant@univ-lorraine.fr,

Chromium (Cr) exists in several oxidation states; the most common being hexavalent Cr (Cr(VI)) and trivalent Cr (Cr(III)). Various industrial activities (e.g. steel mills, tanneries) are associated with significant releases of Cr(III) or Cr(VI) into the environment. The toxicity of Cr(VI) is well established and the mechanisms involved in its cellular uptake and handling quite well documented. In particular, sulfate  $(SO_4^{2-})$  transporters import chromate  $(CrO_4^{2-})$  in prokaryotic and eukaryotic cells because of the structural homology between the two ions. Furthermore, in presence of CrO<sub>4</sub><sup>2-</sup>, bacteria and several ascomycetes express a plasma membrane protein involved in CrO<sub>4</sub><sup>2-</sup> export from the cytoplasm to prevent Cr(VI) toxicity. The reduction of Cr(VI) is also associated with reactive oxygen species (ROS) production. The increase of ROS generates oxidative stress in cell and causes DNA and protein damages. The general consensus considers Cr(III) to be less toxic than Cr(VI), although an increasing number of studies suggests that Cr(III) can also be of concern. Chlamydomonas reinhardtii is a singlecell green alga and is ubiquitous in aquatic environments and temperate soils. C. reinhardtii is a reference model for studying molecular and cellular biology of cell cycle. In this study, we have compared C. reinhardtii cellular responses after exposure to Cr(VI), to Cr(III) or to Cr(VI)/Cr(III) mixture by measuring genes expression, photosynthesis, and ROS production. Global proteins modulations were also measured by proteomic approach to define pathways involved in Cr(III) and Cr(VI) responses.

# CyanoTag: High-throughput protein tagging in photosynthetic bacteria

Perrin, A.J.<sup>1\*</sup>, Dowson, M.<sup>1</sup>, Zhao, G.Z.<sup>2</sup>, Dowle, A.A.<sup>1</sup>, Calder, G.<sup>1</sup> & Mackinder, L.C.M.<sup>1</sup>

<sup>1</sup>University of York, United Kingdom <sup>2</sup>Shandong Normal University, China \*abi.perrin@york.ac.uk

Photosynthetic bacteria are fundamentally important to aquatic ecosystems and global carbon cycling and have exciting potential for use in sustainable bioindustries. They can also be used as a genetically tractable model system to understand aspects of fundamental plant and algal biology. However, much of their basic biology is not yet understood and their genomes still contain large numbers of uncharacterised protein-coding genes. To accelerate our understanding of these essential microorganisms we have developed a high-throughput pipeline for the scarless integration of tags that facilitate protein localisation, expression and interactome profiling in the model cyanobacterium *Synechococcus elongatus* PCC7942.

We have now used this CyanoTag platform to fluorescently label around 20% (>500 proteins) of the *S. elongatus* proteome. Localisation of these proteins via super-resolution imaging has provided exciting novel biological insights into a diverse range of processes including the regulation of photosynthesis and cell division. By analysing our CyanoTag lines with an affinity-purification mass-spectrometry

based approach we have also mapped a preliminary interactome. We are now expanding the CyanoTag library to target the whole *S. elongatus* proteome, and exploring the ways in which this resource may be best used and shared to facilitate novel discoveries within the wider scientific research community.

#### Life of Chlamydomonas reinhardtii in a nature-like environment

Vuong, T.<sup>1\*</sup>, Kurtoglu, E.<sup>1</sup>, Chowdhury, S.<sup>2</sup>, Mukherji, R.<sup>2</sup>, Stallforth, P.<sup>2</sup> & Mittag, M.<sup>1</sup>

<sup>1</sup>Matthias Schleiden Institute of Genetics, Bioinformatics and Molecular Botany, Friedrich Schiller University Jena, Am Planetarium 1, 07743 Jena, Germany

<sup>2</sup>Department of Paleobiotechnology, Leibniz Institute for Natural Product Research and Infection Biology, Hans Knöll Institute (HKI), Beutenbergstraße 11a, 07745 Jena, Germany \*trang.vuong@uni-jena.de

The biflagellate unicellular green alga *Chlamydomonas reinhardtii* serves as a model organism for studying different biological processes such as the influence of light and temperature. In the laboratory, this alga is cultured axenically in liquid or on solid medium. In nature, it is usually found in moist soil. To study the growth of *C. reinhardtii* in a nature-like environment, we have introduced 3-D structured components using glass beads. *C. reinhardtii* vegetative cells were grown in a light-dark cycle at 23 °C. Interestingly, the algal growth is enhanced in the 3-D environment compared to a pure liquid culture. Furthermore, in the 3-D environment, *C. reinhardtii* grows within a broad range of pHs from 5 to 9, while it can not grow at low pH (5) in liquid culture. Transcriptome data of cells grown in 3-D environment in comparison with pure liquid show major differences. Especially the photosynthesis related transcriptome is highly upregulated while the flagellar transcriptome is strongly downregulated. This goes hand in hand with alterations in the length of the algal flagella in 3D environment as shown by immunolocalization studies. Consequently, the mating efficiency of *C. reinhardtii* gametes is also less efficient in 3-D environment. These data give us important insights into the molecular mechanisms that govern the life of *C. reinhardtii* under natural conditions.

### Impacts of the loss of a histone post-translational mark on the physiology of the pennate diatom *Phaeodactylum tricornutum*

Zarif, M.\*, Groisillier, A., Tirichine, L. & Duc, C.

Nantes Université, CNRS, US2B, UMR 6286, F-44000 Nantes, France \*mhammad.zarif@univ-nantes.fr

Enhancer of zeste (EZH) is a histone methyl transferase and the catalytic subunit of the polycomb repressive complex 2 (PRC2). It is responsible for the deposition of the trimethylation on the lysine 27 of the histone H3 (H3K27me3) in various organisms and notably in the pennate diatom *Phaeodactylum tricornutum*. This diatom displays four morphotypes (fusiform, oval, triradiate and cruciform). Depletion of the H3K27me3 epigenetic mark triggers changes in cell morphology and gene expression in *P. tricornutum*. However, the role of H3K27me3 in *P. tricornutum* physiology remains unclear.

I observed that growth of EZH knockout lines in various morphotypes is slower compared to wild-type lines. Therefore, we carried out a comprehensive study to understand the factors behind growth impairment in various morphotypes. The PSII (PhotoSystem II) efficiency was measured with a Pulse Amplitude Modulation (PAM) fluorometer and the relative content of chlorophylls a and c was measured in extracts using spectrophotometry. Finally, in the context of the ongoing climate change and

the resulting rise in sea temperatures, we assessed the stability of morphotypes and their growth under optimal and heat stress temperatures.

### SYMPOSIUM 07 "Genomics technologies in algae"

# Characterization of a uranium-tolerant microalga of the genus *Coelastrella* with high potential for the remediation of metalpolluted waters

Beaulier, C.\*, Dannay, M., Devime, F., Bourguignon, J., Alban, C. & Ravanel, S.

Laboratoire de Physiologie Cellulaire & Végétale, Univ. Grenoble Alpes, INRAE, CEA, CNRS, 38000 Grenoble, France

\*camille.beaulier@cea.fr

Uranium (U) pollution of terrestrial and aquatic ecosystems poses a significant threat to the environment and human health because this radionuclide is chemotoxic. Some microalgae are capable of biosorbing large quantities of metals and may be of biotechnological interest for the treatment of polluted waters (phycoremediation). Here, we isolated a novel green microalga strain of the genus *Coelastrella* that is much more tolerant to U than *Chlamydomonas reinhardtii* and *Chlorella vulgaris*. *Coelastrella* is able to accumulate U very rapidly and, then, progressively release it into the medium, behaving as an excluder to limit the toxic effects of U. The ability of *Coelastrella* to capture U is remarkably high, with up to 600 mg U sorbed per g dry biomass. *Coelastrella* is able to grow in natural metal-contaminated waters from a reclaimed U mine and to capture 25-55% of contaminating U. Additionally, algae grown in metalpolluted waters accumulate lipid droplets. The isolated *Coelastrella* sp. is a very promising microalga for the remediation of polluted waters with valorization of algal biomass that accumulates neutral lipids. Transcriptome analysis is ongoing to identify molecular actors involved in U tolerance mechanisms in *Coelastrella* sp.

#### Mutation Frequency of *Chlamydomonas reinhardtii* Using CRISPR-Cas9 Ribonucleoprotein Is Tripled by Employing *Agrobacterium* Originated Nuclear Localization Signal

Choi, H.I.<sup>1\*</sup>, Le, T.T.<sup>1,2</sup>, Kim, J.W.<sup>1,2</sup>, Kim, H.-S.<sup>1,2</sup>, & Lee, Y.J.<sup>1,2</sup>

<sup>1</sup>Cell Factory Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Daejeon, South Korea 34141

<sup>2</sup>Department of Environmental Biotechnology, KRIBB School of Biotechnology, University of Science and Technology (UST), Daejeon, South Korea 34113

\*superstitione@kribb.re.kr

Microalgae are attractive sources of CO<sub>2</sub>-derived sustainable products. So far, their real-world applications have been hindered because of the low productivities of the biomass itself and the products therefrom. In this context, genetic engineering tools have been intensively used to boost the production performances of microalgae up to commercializable levels. Specifically, the CRISPR-Cas9 ribonucleoprotein (RNP) system has been considered the most promising technical candidates due to its precise gene editing ability. However, Cas9 RNPbased gene editing attempts have been frustrated as a consequence of the low mutation frequency that is mainly caused by the low translocation efficiency of

the extracellularly provided RNP complex into the microalgae nucleus. Herein, we developed a highly efficient

CRISPR-Cas9 RNP system, which was rationally designed to improve the translocation of the CRISPR-Cas9 RNP complex through the nuclear membrane, thereby raising the mutagenesis frequency. To this end, the nuclear localization signal (NLS) of Cas9 protein was substituted by a plant-infecting bacterium-originated NLS, whose binding affinit 40 large T antigen-derived NLS. As a result of the mutation using the designed Cas9 RNP, the overall targeted mutagenesis frequency was dramatically improved to  $1.12 \times 10^{-5}$ , demonstrating 3-fold improvement compared to the mutagenesis attempt with the Cas9 fused with the conventional NLS (i.e.,  $0.37 \times 10^{-5}$ ). We expect that this highly efficient and precise gene editing machinery will facilitate the realization of microalgal industries by alleviating the difficulty in microalgal mutagenesis.

# An individual database created from genome and transcriptome data allows for proteomic analysis of *Dunaliella* in response to hyperosmotic conditions

Keil, L.\*, Mehlmer, N., Garbe, D. & Brück, T.

Werner Siemens-Chair of Synthetic Biotechnology, Technical University of Munich, Lichtenbergstr. 4, D-85748 Garching, Germany \*Linda.Keil@tum.de

Algae based bioprocesses can contribute to greenhouse gas remediation, while producing value adding compounds. One promising solution is the direct  $CO_2$  fixation in algae-based glycerol, which can be converted into 'green chemicals' to replace fossil-based ones, such as polyacrylonitrile and carbon fibers. The marine microalgae *Dunaliella tertiolecta* is reported to accumulate high amounts of intracellular glycerol upon exposure to high salt concentrations.

Proteomic studies of the intracellular response to hyperosmotic condition changes are only available for *D. salina*. To enhance the database and to establish *D. terticolecta* as a reference for future analysis of unspecified *Dunaliella* species in our research group, insights into the algae cell's behavior should be revealed by modern systems biology methods. Due to a lack of a reference genome, which is required for further studies, the genome of *D. tertiolecta* was analysed in-house with the advanced PacBio sequencing method. An individual database was designed by combining the genome with transcriptome data obtained before and after a substantial increase in salt concentration. The database allowed not only the detection of down- or upregulated proteins, but also to identify phosphorylated proteins as quick response to hyperosmotic stress.

Actual data show that the developed database results in more hits compared to published databases e.g. from *D. salina*. Furthermore, the results of this study will provide novel insights into hyperosmotic shock responses of the halophilic alga *D. tertiolecta*. With establishing *D. tertiolecta* as reference, the path is opened to transfer the methods onto unspecified *Dunaliella* strains.

# Genetic tool kits in *Pyropia yezoensis* against biotic and abiotic stresses

Kim, Y.\*, Kim, R-W. & Kim, G.H.

Department of Biology, Kongju National University, Kongju 314-701, Korea yong2@kongju.ac.kr

Many organisms share a set of genes that respond to various stressors, both biotic and abiotic. These genetic elements have been termed as an organism's genetic tool kit against stress. The three main stressors that cause severe economic damage to Pyropia farms are oomycetes infections by Olpidiopsis spp. (Olpidiopsis disease) and Pythium porphyrae. (red rot disease) and due to the depletion of nutrients in seawater (chlorosis). How Pyropia responds to these stressors is not well known and the similarities in genetic response needs investigation. We investigate the molecular response of *Pyropia vezeonsis* to each stressor through RNA-seq analysis. We compared and interpreted the transcriptome profiles of P. yezoensis response to these three stressors in terms of the stress defense mechanism (genetic tool kit genes), especially specific response genes (Heat-shock proteins and serine protease) together with histochemical observations. We found that many genes were differential expressed during stress (1,445 genes in *Olpidiopsis* disease;769 in red-rot-disease; and 880 genes in chlorosis). Genes with a ≥4-fold decrease in expression were most prevalent in the chlorosis condition. Nine genes increased and seven decreased expression across all stress conditions, and there was also a common decrease in photosynthesis-related genes. Heat shock proteins and serine proteases were only upregulated during oomycetes infection. Taken together, these findings suggest that P. vezeonsis has a plant-like stress response system. It is believed that it responds to stress by decreasing the expression of genes in response to abiotic stresses to survive nutrient poor conditions, and increases the expression of genes in response to biotic stresses (oomycete disease) to counteract the causative agent.

## Identifying novel marine microalgal enzymes for industrial applications using omics approaches

Marec, G.<sup>1\*</sup>, Lukicheva, S.<sup>2</sup>, Bilcke, G.<sup>1,2</sup>, De Cock, L.<sup>3</sup>, Devreese, B.<sup>3</sup>, Vyverman, W.<sup>1</sup>, Vandepoele, K.<sup>2</sup>, Asselman, J.<sup>4</sup> & Sabbe, K.<sup>1</sup>

<sup>1</sup>Protistology and Aquatic Ecology, Ghent University, Belgium

<sup>2</sup>Vlaams Instituut voor Biotechnologie, Center for Plant Systems Biology, Belgium

<sup>3</sup>Laboratory for Microbiology, Department of Biochemistry and Microbiology, Ghent University, Belgium

<sup>4</sup>Blue Growth Research Lab, Ghent University, Belgium

\*Guillaume.Marec@UGent.be

Enzymes have become increasingly important in the production of agronomic, food, and feed products due to their non-toxic and biodegradable properties, making them a desirable alternative to standard chemicals. Therefore, there is a growing demand for new and more efficient enzymes. Microalgae from the marine environment represent an interesting source of new enzymes, as they have been much less studied and often tolerate variable and extreme conditions (such as pH, low and high temperature, high salinity...). Diatoms, in particular, are a promising candidate for the discovery of new enzymes due to their complex and chimeric genomes. Our work aims to identify new enzymes of industrial interest in diatoms and evaluate their potential use in industrial applications.

A first selection, based on enzymatic tests and known habitat preferences, was performed to select the candidates for proteomic screening. Several strains of *Cylindrotheca closterium* from different thermal niches were chosen for whole genome resequencing, and an estuarine strain of *Cyclotella meneghiniana* was selected for whole-genome sequencing. RNAseq and proteomics will be used for genome annotation. This approach should allow identifying novel enzymes with unique features, such as temperature tolerance.

We will present the first results of initial analyses of *Cylindrotheca closterium* using enzyme screening and shot-gun proteomics, which allowed identifying candidate enzymes of potential interest.

# Towards the clarification of diatom lipid catabolism: an *in silico* genome and transcriptome mining for *Phaeodactylum tricornutum* lipases

Murison, V.<sup>1\*</sup>, Hérault, J.<sup>1</sup>, Schoefs, B.<sup>2</sup>, Marchand, J.<sup>2</sup> & Ulmann, L.<sup>1</sup>

<sup>1</sup>BiOSSE: Biology of Organisms, Stress, Health, Environment, Département Génie Biologique, Institut Universitaire de Technologie, Le Mans Université, F-53020 Laval, France <sup>2</sup>BiOSSE: Biology of Organisms, Stress, Health, Environment, UFR Sciences et Techniques, Le Mans Université, F-72085 Le Mans, France \*victor.murison@univ-lemans.fr

Under abiotic stress conditions, diatoms such as *Phaeodactylum tricornutum* reorient the carbon metabolism toward neutral lipid accumulation through a combination of *de novo* synthesis and recycling. How these lipids are used when cells recover from stress is not well characterized. Based on mechanisms found in other eukaryotes, consumption of neutral lipids could occur by lipolysis with direct lipid degradation in the lipid droplet or/and through lipophagy with bulk lipid droplets degradation by autophagy in vacuoles. Both mechanisms suppose the action of lipases, the enzymes that catalyze triacylglycerol hydrolysis.

This communication will consist in presenting the results of a screening of the annotated genome of P. *tricornutum* designed to identify lipases and select those that are probably active in either mechanisms. An *in silico* search for conserved motifs highlighted a total of 57 putative lipases. Further characterization included subcellular localization prediction showing that three enzymes could be vacuolar and that 36 of them had palmitoylation sites allowing a possible transient association to lipid droplets.

To investigate the *in vivo* activity of the putative lipases, three publicly available transcriptomic datasets from *P. tricornutum* under a nitrate starvation were examined. The transcripts encoding nine candidate lipases were found to be highly regulated under this standard stress condition. The 3D structure of this subset of proteins was predicted from their sequence using Alphafold2 and showed that eight of them had potentially active structures. Finally, by combining the results of these analyses, two enzymes were highlighted as targets for functional studies.

# The effect of ocean warming and CO<sub>2</sub> enrichment on *Ecklonia radiata*: investigating molecular responses

Wynn, O.J.\*, Britton, D., Beardall, J., Bridle, A.R. & Hurd, C.L.

Institute for Marine and Antarctic Studies, University of Tasmania, Tasmania, Australia \*Olivia.wynn@utas.edu.au

Kelps form the foundations of diverse and productive coastal ecosystems but increasing carbon dioxide (CO<sub>2</sub>) concentrations and ocean temperatures pose a threat to their survival and ecological function. *Ecklonia radiata* is the most widespread and abundant habitat forming kelp in Australia and New Zealand, forming extensive forests and persists in seawater temperatures ranging from  $\sim 7 - 26$  °C. The mechanisms underlying the physiological responses of *E. radiata* to the interactive effects of CO<sub>2</sub> enrichment and warming are poorly understood. This study investigates the molecular processes underlying physiological acclimation to better understand the potential responses of macroalgae to near-future climate change scenarios. Here we compared the physiological and gene expression responses of

*E. radiata* to three different temperatures (6 °C, 16 °C, and 26 °C), and ambient and elevated CO<sub>2</sub> conditions (RCP 8.5).

At 16 °C under future  $CO_2$  conditions, relative to ambient  $CO_2$  conditions, a clear upregulation of genes encoding for photosynthetic function was observed with no down regulation of the carbon dioxide concentrating mechanism, which could explain no change in growth. Following exposure to 26 °C relative to 16 °C, genes encoding ribosomal proteins and autophagy-related proteins were upregulated, suggesting high protein turnover, to replace proteins damaged due to high temperature stress. Furthermore, genes encoding fatty acid metabolism, photosynthetic function, and antioxidant production were upregulated as various defence mechanisms. This study represents a significant step forward in understanding the underlying mechanisms in response to ocean global change and enhances our ability to predict the outcome of some macroalgal species.

### SYMPOSIUM 08 "Algal photosynthesis, carbon fixation and respiration"

### Multi-scale temporal variability in MPB biomass and production in diatom- and euglenophyte dominated estuarine biofilms

Bas, D.A.<sup>1,2\*</sup>, Sabbe, K.<sup>2</sup>, van der Wal, D.<sup>3</sup>, Cox, T.<sup>1</sup> & Meire, P.<sup>1</sup>

<sup>1</sup>University of Antwerp, Department of Biology, ECOSPHERE, Belgium <sup>2</sup>Ghent University, Department of Biology, Protistology & Aquatic Ecology (PAE), Belgium <sup>3</sup>Royal Netherlands Institute of Sea Research (NIOZ), Department of Estuarine & Delta Systems (EDS), The Netherlands \*dorian.bas@uantwerpen.be

Microphytobenthos (MPB) biomass is characterized by pronounced temporal variability at multiple scales, in relation to tidal and diurnal, spring-neap, seasonal and interannual cycles. Field sampling but also airborne remote sensing is not able to fully capture this variability. We installed optical sensors to measure Normalized Difference Vegetation Index (NDVI) on estuarine intertidal flats at three locations (marine, brackish and freshwater) along the salinity gradient of the Schelde estuary, and monitored MPB surface biomass from October 2020 to september 2022 every ten minutes.

We found a similar correlation between NDVI and chlorophyll a in the freshwater and brackish intertidal flats. In the freshwater biofilms, which were dominated by euglenophytes, MPB biomass reached its highest values (NDVI of up to 0.69 corresponding to 260 mg Chl a/m<sup>2</sup>) during summer. In contrast, the brackish MPB communities were dominated by epipelic diatoms and showed a distinct biomass peak (NDVI up to 0.42 corresponding to 159 mg Chl a/m<sup>2</sup>) in winter (December-February, and to a lesser degree early spring). Both MPB communities showed rapidly fluctuating biomass during spring and summer. Vertical up- and downward migration during emersion was more gradual in the euglenophyte biofilms, but was very rapid in the diatom-dominated biofilms. In the marine station, MPB biomass was much lower, and showed little variability at all temporal scales. We will discuss the biomass patterns outlined above, and indicate how including high-resolution temporal variation in biomass can be used for benthic primary production modeling.

# Quantifying Rates of Growth, Erosion and Dislodgment of Cultivated Kelp in the UK

Canvin, M.C.<sup>1,2\*</sup>, Smale, D.A.<sup>1</sup> & Moore, P.J.<sup>2</sup>

<sup>1</sup>The Marine Biological Association of the United Kingdom, UK <sup>2</sup>Newcastle University, UK \*maxcan@mba.ac.uk

Kelp-dominated habitats are not traditionally considered Blue Carbon ecosystems. However, due to high rates of primary production and carbon export, they may serve as important donors in natural carbon sequestration. While seaweeds, including kelp, have been historically hand-harvested in the UK, cultivation of seaweeds for various products (e.g. nutraceuticals and alginate) is an emerging industry. Kelp farming may also provide additional ecosystem benefits and services, such as enhance fisheries habitat or elevate carbon sequestration, but the evidence base remains poor. As such, quantifying carbon dynamics in and around kelp farms is crucial to determining their viability as a Blue Carbon climate change mitigation strategy.

Over the 6 month growing season, the growth and loss of cultivated *Saccharina latissima* biomass was monitored at an integrated multi-trophic kelp and mussel farm in Cornwall, UK, to quantify the accumulation of carbon as biomass and the release of particulate organic carbon (POC). A modified hole-punch method was used to monitor both meristematic growth and distal erosion of the blades, and individual plants were tagged to quantify dislodgement rates. Preliminary results demonstrate the pronounced seasonality in productivity and POC dynamics. Next steps include quantifying the release of dissolved organic carbon (DOC) by cultivated *S. latissima* and quantifying storage of kelp-derived carbon within sediments associated with the farm. This will provide a holistic overview of the carbon dynamics of kelp farms and the capacity for carbon sequestration in sediments below farms.

# Using different light-emitting diodes (LEDs) in *Ulva ohnoi* (Ulvales, Chlorophyta) cultures. Implications in IMTA-RAS systems

García-Cardesín, E.<sup>1</sup>, Del Olmo, G.<sup>2</sup>, Pintado, J.<sup>2</sup> & Cremades, J.<sup>1\*</sup>

<sup>1</sup>Interdisciplinary Center for Chemistry and Biology, University of A Coruña, Spain <sup>2</sup>Institute of Marine Research (IIM-CSIC), Galicia, Spain \*javier.cremades@udc.es

Light-emitting diode (LED) technology has opened new doors to indoor algae aquaculture by helping to greatly reduce production costs and even improve their growth rate, proximal composition and, in IMTA-RAS systems, modify the biofiltration capacity or the microbiome associated with algae and their possible probiotic effects on other trophic levels of these systems.

In this work, the influence of different led lights (blue, red, white and magenta) and intensities on the growth rate, dry matter, elemental composition (CNHS), richness in proteins, pigment content and biofiltration capacity were tested for the first time in *Ulva ohnoi* M. Hiraoka & S. Shimada (Ulvales, Chlorophyta), one of the most promising species for use in IMTA-RAS fish-macroalgae systems. The experiences were carried out using a culture medium with high levels of N and P to simulate the effluent of the fishes. The best results in almost all the aspects studied in *U. ohnoi* were obtained with blue light, which also presents the lowest energy consumption for the same incident light intensity on the surface of the experimental plates.

# Peculiarities of diatom ECS allow *in vivo* measurements of the transthylakoidal proton gradient ( $\Delta pH$ )

Croteau, D.\* & Bailleul, B.

CNRS, Chloroplast Biology and Light-sensing in Microalgae-UMR7141, France croteau@ibpc.fr

The transthylakoidal electrochemical proton gradient ( $\Delta \mu_{H^+}$ ) drives ATP production in photosynthesis. It comprises a proton concentration gradient ( $\Delta pH$ ) and an electric field ( $\Delta \psi$ ).

The  $\Delta pH$  is often described as the "master regulator" of photosynthesis in the green lineage. Methodologies have been proposed to measure  $\Delta pH$  in plants, which are based on transient ECS inversion after a light-dark transition, but most marine microalgae, such as the diatom *Phaeodactylum tricornutum*, do not exhibit those inversions. In contrast, the regulatory roles of the  $\Delta \psi$  are not as clear, but its relative variations are measured straightforwardly as they induce a shift in pigments absorption spectra due to the Stark effect. This probe is called the linear electrochromic shift (ECS). Diatoms also display a quadratic ECS probe, and this peculiar feature has been used to measure the <u>absolute</u>  $\Delta \psi$  in diatoms (Bailleul et al., 2015, Nature). Another spectroscopic method developed by Joliot & Joliot (2008, BBA) suggests that the  $\Delta \mu_{H+}$  threshold for the activation of ATPsynthase can be used as reference to infer relative values of  $\Delta \mu_{H+}$ . Therefore, by combining the two described methods, it is theoretically possible to measure the  $\Delta pH$  from estimations of  $\Delta \mu_{H+}$  and  $\Delta \psi$ , in diatoms. We are currently testing this approach by investigating how the total  $\Delta \mu_{H+}$  and its partitioning varies in the presence of different uncouplers and during the relaxation of nonphotochemical quenching after high light stress. The coherency of our preliminary results between the methods and with the expected theoretical values are encouraging.

### Measurements of photosynthesis in mixture reveal the role of nutrients and allelopathy in the competition between dinoflagellates and diatoms

Gain, G.<sup>1\*</sup>, Peltekis, A.<sup>1</sup>, Mével, G.<sup>1</sup>, Fontana, A.<sup>2</sup> & Bailleul, B.<sup>1</sup>

<sup>1</sup>UMR7141 CNRS – IBPC – Biologie du Chloroplaste et Perception de la Lumière chez les Microalgues, 13, rue Pierre et Marie Curie, 75005 Paris, France <sup>2</sup>Istituto di Chimica Biomolecolare (ICB)-CNR, Via Campi Flegrei 34, 80078 Pozzuoli, Napoli, Italy.

\*gain@ibpc.fr

In the Ocean, the distribution of species depends on many factors including turbulence and nutrient availability. Allelopathy, the release of allelochemicals detrimental to the physiology of competitors, is also believed to influence the structuration of phytoplankton assemblages. A new method based on ElectroChromic Shift (ECS) allows the analysis of the photosynthetic performance of several microalgae within a mixture. ECS behaves as an "internal voltmeter" and shows different spectral signatures in each photosynthetic clade; it allows therefore to reveal allelopathic interactions targeting photosynthesis. We observe that the photosynthesis and growth of the diatom *T. pseudonana* are arrested in the presence of the dinoflagellate *A. carterae*. This inhibition depends on the production and secretion by the dinoflagellate of a secondary metabolite of the amphidinol family, which suppresses the proton motive force across the diatom's thylakoids. By using the diatom's photosynthesis as a proxy, we also investigate the production/secretion of the amphidinol (AM) as a function of the nutrient availability and stage of the growth of *A. carterae*. Three distinct phases occur: (i) during the exponential phase,

amphidinols are not produced; (ii) as soon as stationary phase is reached, the concentration of intracellular AM starts and from then increases continuously with time and (iii) later in the stationary phase, significant amounts of AM are secreted. The production of AM per cell is further increased in conditions of phosphate depletion. This new methodology reveals the potential of photosynthesis as a tool for stress diagnostic and environmental monitoring in the Ocean.

### Impact of sulfur limitation on marine microalgae cell composition and photosynthesis

Minio, M., Spinelli, S. & Gerotto, C.\*

Laboratory of algal and plant physiology, Dept. of Life and Environmental Sciences, Università Politecnica delle Marche, Italy \*c.gerotto@univpm.it

Sulfur is an essential macroelement in cells. It is acquired by cells as sulfate and assimilated, through a highly demanding reductive process, as sulfide into cysteine. In addition to the Sharboring amino acids cysteine and methionine, S is found in various other cellular components, like sulfolipids and Fe-S clusters. It also plays a number of functional roles in cells, including the redox regulation of enzymes and electron transport. Despite such central roles, most of our knowledge on S metabolism is focused on plant species, while in microalgae it is still fragmentary, particularly because of their huge phylogenetic diversity. We are investigating how different marine phytoplanktonic species acclimate to low sulfate availability. We characterized the photosynthesis of microalgae grown in limiting or replete sulfate conditions by means of *in vivo* chlorophyll fluorescence analyses. Elemental analysis allowed the quantification the cell quota of macroelements and investigate shifts in the elemental ratios as a response to sulfate availability, while Fourier Transformed Infrared spectroscopy depicted the macromolecular composition of the algal cells as a function of growth conditions. Results suggest that the cultures successfully acclimated to the low sulfate growth conditions by prioritizing the allocation of available resources to core processes, like photosynthesis, while reducing their growth and modulating the cell composition in a species-specific manner.

## Diel photosynthesis rhythm in diatoms: evidence of endogenous circadian regulation

Guérin, S.1\*, Babin, M.1 & Lavaud, J.1,2

<sup>1</sup>Takuvik, Canada <sup>2</sup>LEMAR-Laboratory of Environmental Marine Sciences, France \*sebastien.guerin@takuvik.ulaval.ca

Diel light cycles punctuate the existence of most terrestrial and marine organisms. The mechanisms regulating the daily rhythms of diatoms, a prominent marine unicellular primary producer, are still unknown, but evidence for an endogenous circadian regulation are multiplying. In this study, we define the features of the photoperiodic rhythm of photosynthetic performance in the model polar diatom *Fragilariopsis cylindrus*, under different spectra, including monochromatic blue and red lights. A polar model was selected because diatoms are exposed to extreme changes in day length at high latitudes. We report a circadian regulation of many aspects of the photochemistry and photoprotection relying on the light-darkness alternation, and likely on the cell division cycle. We additionally characterized a robust endogenous circadian regulation of energy allocation in the photosystem II

reaction centers. Our results also support that most of the robustness of the rhythmic response of photochemistry and photoprotection is mainly attributed, but not only (i.e. red light is important), to the blue wavelengths, which are found dominant at the bottom and underneath sea-ice, the natural habitat of *F. cylindrus*. We hypothesize that fine-tuning the balance between photochemistry and photoprotection along with circadian synchronization allows *F. cylindrus* to make the most of available light to support sustained production and growth across a broad range of photoperiods, and despite low light, and low temperature (ca.  $0^{\circ}$ C).

# Strong heterologous electron sink outcompetes alternative electron transport and elucidates coordination of electron distribution from PSI

Hubáček, M.<sup>1</sup>\*, Wey, L.T.<sup>1</sup>, Kourist, R.<sup>2</sup>, Malihan-Yap, L.<sup>2</sup>, Nikkanen, L.<sup>1</sup> & Allahverdiyeva, Y.<sup>1</sup>

<sup>1</sup>Photosynthetic Microbes, Molecular Plant Biology unit, Department of Life Technologies, University of Turku, Finland

<sup>2</sup>Institute of Molecular Biotechnology, NAWI Graz, BioTechMed, Graz University of Technology, Austria \*michal.hubacek@utu.fi

Improvement of photosynthesis requires a thorough understanding of electron partitioning under both natural and strong electron sink conditions. We applied a wide array of biophysical and biochemical techniques to thoroughly investigate the fate of photosynthetic electrons in the engineered cyanobacterium Synechocystis sp. PCC 6803, heterologously expressing the gene for ene-reductase, YqjM. This recombinant enzyme catalyses the reduction of an exogenously added substrate into the desired product by utilising photosynthetically produced NAD(P)H, enabling whole-cell biotransformation.

Through coupling the biotransformation reaction with biophysical measurements, we demonstrated that the strong artificial electron sink, YqjM, outcompetes the natural electron valve, flavodiiron proteindriven Mehler-like reaction, and cyclic electron transport. These results suggest that ferredoxin-NAD(P)H-oxidoreductase (FNR) is the preferred route for delivering photosynthetic electrons from reduced ferredoxin and the cellular NADPH/NADP+ ratio as a key factor in orchestrating photosynthetic electron flux. These insights are crucial for optimising cellular source/sink balance and harnessing photosynthesis for sustainable bioproduction.

## Optical properties of an aerated sample cell in an open-flow through steady-state gas exchange system

Hupp, J.<sup>1</sup>\*, Eckels, R.<sup>1</sup> & Smillie, I.<sup>2</sup>

<sup>1</sup>LI-COR Biosciences, USA <sup>2</sup>LI-COR Biosciences Ltd., UK \*Jason.hupp@licor.com

Steady-state gas exchange methods determine gas exchange rates from the difference in concentration of a target gas species in an air stream before and after it interacts with a sample. The magnitude of that difference is dependent on the flow rate of air through the cell holding the sample and the sample's exchange rate of the target gas species. In a steadystate measurement extending the observation period does not increase signal magnitude as with a closed-transient gas exchange method.

Steady-state methods have been long applied for leaf-level measurements of photosynthetic carbon assimilation and respiration. Similar methods can be applied to samples in liquid suspension, such as algal cultures or aquatic macrophytes. Precision of existing technologies for carbon dioxide measurement set bounds on the minimum amount of sample material necessary. The highest precision technologies employed in gas exchange systems make gas phase measurements, necessitating some means of driving the gas-liquid equilibrium with the sample volume (active aeration). Measurements are typically made in some three-dimensional volume of sufficient size to ensure enough sample for detection and to accommodate the gas phase equilibration method. This volume represents a complex light environment that must be understood to make sense of measured assimilation rates. Here we describe the light environment in a commercial gas exchange chamber designed to measure liquid samples. Data from direct measurement and computer simulation are provided describing optical throughput in the cell, light uniformity, and the effects of the aeration scheme used to drive gas phase equilibration.

# Directed evolution of an extremophilic red microalga *Cyanidioschyzon merolae* leads to stable adaptation to high concentrations of heavy metals

Marchetto, F.<sup>1\*</sup>, Abram, M.<sup>1</sup>, Lebiedzińska-Arciszewska, M.<sup>2</sup>, Śliwińska, M.A.<sup>3</sup>, Szymański, J.<sup>3</sup>, Góral, T.<sup>4</sup>, Santaeufemia, S.<sup>1</sup>, Solymosi, D.<sup>5</sup>, Adamska, D.<sup>6</sup>, Palatini, J.<sup>6</sup>, Allahverdiyeva-Rinne, Y.<sup>5</sup>, Więckowski, M.R.<sup>2</sup> & Kargul, J.<sup>1</sup>

<sup>1</sup>Solar Fuels Laboratory, Centre of New Technologies, University of Warsaw, 02-097 Warsaw, Poland.
<sup>2</sup>Laboratory of Mitochondrial Biology and Metabolism, Nencki Institute of Experimental Biology PAS, Warsaw, Poland.
<sup>3</sup>Laboratory of Imaging Tissue Structure and Function, Nencki Institute of Experimental Biology PAS, 02-093 Warsaw, Poland.
<sup>4</sup>Cryomicroscopy and Electron Diffraction Core Facility, Centre of New Technologies, University of Warsaw, 02-097 Warsaw, Poland.
<sup>5</sup>Molecular Plant Biology Unit, Department of Life Technologies, University of Turku, Turku FI-20014, Finland.
<sup>6</sup>Next-Generation Sequencing Core Facility, Centre of New Technologies, University of Warsaw, 02-097 Warsaw, Poland.
\*f.marchetto@cent.uw.edu.pl

*Cyanidioschyzon merolae* is an extremophilic red microalga of the *Cyanidiales* order which naturally thrive in extreme environments of volcanic origin. It tolerates moderately high temperatures (up to 56°C), extremely low pH (0.05-4) and the presence of heavy metals. This phototroph is not only considered as a living fossil but also an evolutionary link between cyanobacteria and green lineage as demonstrated by the hybrid structure and function of the photosynthetic apparatus.

We have recently shown that *C. merolae* cells exposed, without preadaptation, to Ni concentrations above 3 mM (4 orders of magnitude higher than the limit set by World Health Organization) show a significant decrease in cell growth and pigment content in conjunction with the inhibition of photosynthetic apparatus and increased production of reactive oxygen species (ROS). Moreover, exposure of cells to 3- and 6-mM Ni leads to the modifications of the chloroplast ultrastructure including the formation of prolamellar bodies and alteration of the thylakoid organization. Using an adaptive laboratory evolution approach, a novel, fully viable and metabolically competent strain of *C. merolae* (dubbed 10NiLAD) has been obtained which thrives at 10 mM Ni. The 10NiLAD cells display growth

rate, photosynthetic parameters and ROS levels comparable to the control. To investigate the putative changes in the organization of the photosynthetic apparatus in the novel high Ni-adapted strain, both photosystems were purified to homogeneity and subjected to cryo-EM analysis. The physiological and structural data allowed us to propose several molecular mechanism of longterm Ni adaptation of this fascinating volcanic microalga.

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# Targeted and non-targeted metabolomics of *Heterocapsa cf. bohaiensis* under Nickel and Iron high concentration and its photophysiology

Meriot V.<sup>1,5</sup>\*, Herbette G.<sup>2</sup>, Garayev E.<sup>3</sup>, Coulombier N.<sup>4</sup>, Le Dean L.<sup>5</sup>, Jauffrais T.<sup>5</sup> & Lebouvier N.<sup>1</sup>

<sup>1</sup>ISEA EA7484, Univ. Nouvelle-Calédonie, 98851 Nouméa, NC, France

<sup>2</sup>CNRS, Centrale Marseille, FSCM, Spectropole, Aix Marseille Université, Campus de St Jérôme-Service 511, 13397 Marseille, France

<sup>3</sup>Aix Marseille Univ, CNRS 7263, IRD 237, Avignon Université, IMBE, 27 Blvd Jean Moulin, Service of Pharmacognosy, Faculty of Pharmacy, 13385 Marseille, France

<sup>4</sup>ADECAL Technopole, 1 Bis Rue Berthelot, 98846 Nouméa, NC, France

<sup>5</sup>Ifremer, UMR Entropie, 101 Promenade Roger Laroque, 98897 Nouméa, NC, France

\*vincent.meriot@etudiant.unc.nc

Microalgae are able to adapt and to synthesize valuable compounds in response to abiotic stresses such as temperature, UV-radiation or metallic trace elements. We studied this faculty of adaptation by assessing the impact of high metal concentrations on Heterocapsa cf. bohaiensis, a newly isolated dinoflagellate from the New Caledonian coastal water rich in metals. We cultivated H. cf. bohaiensis in continuous culture mode using 10L photobioreactors and exposed the algae to high concentrations (10<sup>-</sup>  $^{3}M$ ) of nickel (Ni<sup>2+</sup>) and/or iron (Fe<sup>2+</sup>). We then followed its photosynthetic efficiency using Pulse Amplitude Modulated chlorophyll fluorometry and its metabolome with targeted (HPLC-UV-DAD, GC-MS) and non-targeted (LC-MS<sup>2</sup> and NMR) approaches. The photosynthetic measurements indicate that H. cf. bohaiensis is tolerant to  $Ni^{2+}$  but sensitive to  $Fe^{2+}$  high concentrations. In presence of  $Fe^{2+}$ , Fv/Fm and rETRm decreased from 0.62 to 0.47 and from 156 to 102, respectively. The JIP-tests (i.e. the fast chlorophyll fluorescence transient) suggest that the reduction of photosynthesis in response to Fe<sup>2+</sup> is due to a disruption in the electron transport chain rather than a defect in the PSII light absorption and trapping which are on the contrary enhanced by  $Fe^{2+}$ . The metabolomics analyses highlight the variation of the main dinoflagellate pigments, Chlorophyll c and a, peridinin, diadinoxanthin and diatoxanthin in response to time and metals and give a first glimpse on the complex metabolomic response to metal stress. These results bring new knowledge on this species and on the impact of nickel and iron on the microalgae photosynthetic pathway and metabolomic responses.

## Effect of Rare Earth Element (gadolinium) exposure on microphytobenthos photophysiology

Mohamed Dahlan, M.F.\*, Retif, J., Zalouk-Vergnoux, A., Méléder, V. & Poirier, L.

Nantes Université, Institut des Substances et Organismes de la Mer, ISOMer, UR 2160, F-44000 Nantes, France

\*faazil.mohamed-dahlan@etu.univ-nantes.fr

The global popularity of Rare Earth Elements (REEs), driven by the proliferation of high-tech applications and the development of clean energy, is disrupting natural biogeochemical cycle. One of them, the gadolinium (Gd), has been used for more than 30 years as a contrast agent for magnetic resonance imaging in hospital environment, and little retained by wastewater treatment plants. Consequently, it appears to be one of the most ubiquitous contaminants in aquatic environments of highly populated areas and advanced in terms of the health system. Since then, other observations of contamination of aquatic environments by anthropogenic REEs have been reported, such as lanthanum (La) and samarium (Sm) enrichments are sensitive to this pollution. Bioaccumulation of REEs has been demonstrated by planktonic species, algae, bivalves and various fish species. However, little is known about the impact of these REEs on their physiology.

The objective of this study is to investigate the impacts of Gd, possibly accumulated in the sediment, on the ecophysiological responses of benthic primary producers, *i.e.* the microphytobenthos. After 5 days of acclimation with 12:12 day:night photoperiod at 50  $\mu$ mol.photon.m<sup>-2</sup>.s<sup>-1</sup> and tidal of 9:3 hours of emersion:immersion, the microphytobenthos was exposed to two Gd concentrations: 9 cores have been exposed to 1500  $\mu$ g/L, 9 others to 15  $\mu$ g/L and 9 were used as control. During the exposure for 8 days, pigment changes have been examined by high performance liquid chromatography (HPLC) and their photosynthetic capacity by PAM (Pulse Amplitude Modulation) fluorimetry measurements at D0, D3, D6 and D8.

# Benthic diatoms growth in artificial hydrogels to mimic their natural habitat and to study their physiological properties

Rizzo, A.\* & Jesus, B.

Institut des Substances et Organismes de la Mer–ISOMer UR 2160, Faculté des Sciences et des Techniques, Université de Nantes, 44322 Nantes, France \*arianna.rizzo@univ-nantes.fr

Benthic diatoms colonizing intertidal mudflats –known as microphytobenthos (MPB)– form extensive biofilms at the sediment surface. *Raphideae* diatoms in the MPB can move vertically through the sediment, following mainly the tidal cycles but also as a photoregulation mechanism. They migrate upward (at low tide and low light) and downward (at high tide and high light).

This mechanism has been investigated in freshly collected samples, with a mix of different species, but not in acclimated laboratory conditions, due to the difficulty of isolating and maintaining benthic diatoms alive. In this study we prepared artificial hydrogels made of natural polymers such as Kappacarrageenan, to mimic the natural mudflat substrate.

This technique allowed to isolate and grow diatoms strains uncommon in algae banks i.e., *Pleurosigma sp.*, *Nitzschia spathulata*, *Gyrosigma balticum*, *Navicula sps*. and to study their physiological properties.

Growth in a 3D hydrogel increased the cell number from two times (*Navicula gregaria*) up to four times more (*G. balticum*) than in liquid medium. We characterized the diatom movement behavior (*Pleurosigma sp., N. spathulata, G. balticum*) in response to low and high light exposure, comparing these results with their physiological properties using fast repetition rate chlorophyll *a* fluorometry.

We observed *N. spathulata* did not migrate away from high light, contrary to *G. balticum* and *Pleurosigma sp.*, showing that *N. spathulata* can cope with higher light exposures. This was confirmed by the FRRf analysis, where *N. spathulata* showed higher NPQ response than *G. balticum* and *Pleurosigma sp.* which migrated downward from HL.

## Feasibility study for kelp plantation in the German Bight: habitat availability and light requirements of *Laminaria hyperborea*

Stahl, F.\* & Bischof, K.

Marine Botany, University of Bremen, Leobener Str. NW2, 28359 Bremen, Germany \*fstahl@uni-bremen.de

Besides the much-needed reduction of  $CO_2$  emissions, exploring and implementing carbon removal strategies is necessary to reduce the impact of man-made climate change effects. The sequestration of  $CO_2$  by coastal vegetated ecosystems (CVEs) presents a natural and naturebased solution. One of these CVEs are kelp forests, which are among the most productive and diverse marine ecosystems. Although their  $CO_2$  sequestration potential is still being discussed, kelp forests have been increasingly mentioned within the blue carbon framework in recent years. As kelp forests worldwide are in decline, it is not sufficient to conserve these habitats, but expanding the existing and even establishing new sites is essential. As a baseline study for potential kelp forest expansion around the island of Heligoland and even afforestation measures in the German Bight, we investigated the light requirements of the brown alga *Laminaria hyperborea* and the in situ light climate. Our results point to a compensation irradiance of 30 µmol m<sup>-2</sup> s<sup>-1</sup> for *L. hyperborea* to achieve a positive carbon balance under summer conditions. Combining the kelps minimum light requirement, underwater light attenuation, and bathymetric maps, provides an understanding of habitat requirements based on the physiological needs of *L. hyperborea* and helps to identify suitable afforestation sites within the German Bight.

### SYMPOSIUM 09 "The global carbon-cycle"

#### New experimental setup to investigate marine snow fragmentation

Gesson, M.\*, Le Moigne, F.A.C., Moriceau, B. & Rivière, P.

*UMR6539 – LEMAR, CNRS/UBO/IFREMER/IRD, France* \*maeva.gesson@univ-brest.fr

The ocean's biological carbon pump exports approximately 10 GtC per year and thus constitutes a major process in the carbon cycle and climate regulation. The main process relies on phytoplankton particles aggregating at the surface of the ocean that sediment though the water column: the marine snow. However, before the aggregates reach the seafloor where carbon could be stored for thousands of years, observations show an attenuation of the particle flux in the mesopelagic zone. In addition to the consumption of particles by heterotrophic organisms, part of this attenuation may be due to the aggregates' fragmentation into smaller particles unable to sediment. This phenomenon accounts for half

of the attenuation of carbon flux, making particles fragmentation the main process controlling  $CO_2$  sequestration by the ocean. In this context, we investigated how the oceanic turbulences, which are predicted to increase with climate change, may cause marine snow to fragment.

Testing how different types of phytoplankton aggregates resist - or not - to turbulences, we developed a new experimental setup based on flow-through rolling tanks. We characterized for the first time the turbulences induced by low *vs* high flow input in the rolling tanks. We observed that not only the intensity of the turbulences is different, but the pattern, exposed volume and temporal evolution also are. This characterization allows to set experimental conditions to investigate the turbulence needed to fragment aggregates depending on their composition and morphology. These results will be compared to oceanic turbulences, a first step toward the quantification of particle flux's attenuation in the different layers of the ocean.

## Net primary production facilitated by thermohaline forcings in a stratifed oligotrophic system

Ljubešić, Z.\*<sup>1</sup>, Mihanović, H.<sup>2</sup>, Matek, A.<sup>1</sup>, Mucko, M.<sup>1</sup>, Achterberg, E.P.<sup>3</sup>, Omand, M.<sup>4</sup>, Čižmek, H.<sup>5</sup>, Čolić, B.<sup>5</sup> & Orlić, M.<sup>6</sup>

<sup>1</sup>Department of Biology, Faculty of Science, University of Zagreb, Croatia <sup>2</sup>Institute of Oceanography and Fisheries, Croatia <sup>3</sup>GEOMAR Helmholtz Centre for Ocean Research Kiel, Germany <sup>4</sup>University of Rhode Island, Graduate School of Oceanography, USA <sup>5</sup>Marine Explorers Society 20.000 leagues, Croatia <sup>6</sup>Department of Geophysics, Faculty of Science, University of Zagreb, Croatia \*zrinka.ljubesic@biol.pmf.hr

The oligotrophic Adriatic Sea is in warm period characterized by low productivity driven by strong seasonal stratification that inhibits vertical mixing and nutrient supply to the euphotic layer. These conditions may be disrupted by transient thermohaline forcing that can enhance nutrient flux and create localized hot-spots of net primary production (NPP). Here, we present NPP under the influence of island-trapped waves (ITWs) at Lastovo Island in the Adriatic Sea. During the summer and fall 2022, a set of nine thermistors was deployed on the cliffs at opposite sides of Lastovo Island, at depths between 5 to 45 m. Vertical profiles of temperature revealed diurnal vertical thermocline oscillations ranging up to 25 m. During July, ship-based CTD profiles were measured up to four times a day, and discreet water samples for nutrients collected. Between 16 to 20 July, strong ITWs were associated with nutrient enrichments in surface waters, and large oscillations in the deep chlorophyll fluorescence (Chl F) maximum. After isolating the along-isopycnal Chl F variability, a diurnal cycle in growth and mortality enabled a daily estimation of Gross Primary Production and corresponding losses. The difference between the two provides a measure of NPP and points to the maximum NPP values during the ITW phenomena (18-19 July). These results highlight the role of localized physical phenomena, such as island-trapped waves, in enhancing near-shore primary productivity in the Adriatic archipelago.

# Halimeda tuna in the Adriatic – carbonate production on the microscale of individual segments along the latitudinal and the depth gradients

Nemcova, Y.<sup>1\*</sup>, Orlando-Bonaca, M.<sup>2</sup> & Neustupa, J.<sup>1</sup>

<sup>1</sup>Department of Botany, Charles University, Prague, Czech Republic

<sup>2</sup>Marine Biology Station Piran, National Institute of Biology, Piran, Slovenia \*ynemcova@natur.cuni.cz

Halimeda tuna is the only Halimeda species found in the Mediterranean, and it is an important habitat former. The modular thalli consist of serially arranged calcified segments. Calcification is closely related to photosynthesis, which causes alkalinization of the interutricular space and triggers aragonite formation. Here we present a series of studies describing complex patterns of segment shape plasticity and CaCO<sub>3</sub> content along the Adriatic latitudinal gradient (more than 600 km) and on the depth transect (0.5-9.2 m) at Cape Madona (the northern Adriatic). As a novelty, we applied powerful geometric morphometrics to record size and shape of the segments. In addition, we recorded the position on the thallus and the CaCO<sub>3</sub> content of each segment studied. This allowed us to track slight changes in mineral content on the microscale of the segments. We were able to show that the position of segment on the thallus was the main determinant of its shape and size. The effect of position outweighed shape differences among plants, populations, and regions (along latitude). In addition, segment shape proved to be a significant predictor of its carbonate content. Shape and size of segments were not significantly affected by depth. On the other hand, plants that grew deeper were generally more calcified. The apical and subapical segments contributed to the increase in CaCO<sub>3</sub> content at the deeper sites, while the basal segments did not. This suggests that reniform or oval segments positioned apically or subapically play a key role in calcification of *H. tuna* in Mediterranean ecosystems.

### To improve the accuracy of carbon fixation by macroalgae – Effect of the morphology on carbon and nitrogen content

Sato, Y.<sup>1\*</sup>, Inomata, E.<sup>1</sup>, Tanaka, A.<sup>2</sup> & Nishihara, G.N.<sup>3</sup>

<sup>1</sup>Bio-Resources Business Development Division, Riken Food Co. Ltd., Japan <sup>2</sup>Faculty of Science, University of the Ryukyus, Japan <sup>3</sup>Organization for Marine Science and Technology, Nagasaki University, Japan \*yoi sato@rikenfood.co.jp

Assessing blue carbon potential of macroalgae is under intense investigation. Carbon (C) content provides the strongest evidence of their potential; however, C content can vary spatiotemporally. Estimating the C content of three commercially produced species was our objective. We examined two brown algae, *Undaria pinnatifida* (morphologically differentiated) and *Cladosiphon okamuranus* (morphologically uniform), and one green alga *Ulva prolifera* (morphologically uniform).

We cultivated *U. pinnatifida* at three sites in Iwate Prefecture, Japan. Sporophytes were harvested from the early and late stages of cultivation (i.e., time), and were divided into eight sections (i.e., tip-leaf, mid-leaf, basal-leaf, mid-rib, stipe, sporophylls, stipe of sporophylls, and holdfast). *C. okamuranus* cultured in Okinawa Prefecture was divided into two sections (i.e., tip and bottom parts). *U. prolifera* was collected from land-based cultivation facility in Iwate Prefecture. Wet and dry weights were measured for each section and species. Samples were dried at 80°C for 24 hours and the CN content was measured with an CHN analyzer.

The C content of *U. pinnatifida* varied from 26% in the mid-rib to 34% in the tip-leaf, whereas *C. okamuranus* varied little by section and *U. prolifera* varied little by season (35%). Variation in N content was greater than C in all species, regardless of section and time. We note that estimating total *U. pinnatifida* C content by integrating results derived from each section reveals that C content is overestimated by 40% when compared to commonly used C content values derived solely from the mid-leaf.

## Seaweeds, currents and sedimentation : a hydrodynamic prediction model

Weinberger, F.1\*, Græsdal Rasmussen, T.1, Bouma, T.2 & Karez, R.3

<sup>1</sup>GEOMAR Helmholtz-Centre for Ocean Research, Kiel, Germany <sup>2</sup>NIOZ Royal Netherlands Institute for Sea Research and Utrecht University, Department of Estuarine and Delta Systems, Yerseke, The Netherlands <sup>3</sup>State Agency for Environment Schleswig-Holstein, Kiel, Germany \*fweinberger@geomar.de

Predictions of seaweed drift and sedimentation are needed to model scenarios related with the management of problematic macroalgal blooms, as well as with the potential of macroalgal particles as a carbon sink when they sediment into deeper ocean waters. However, corresponding models that take hydrodynamic traits of seaweeds into account are still lacking. Drag and sedimentation of particles depends on their shape and shape is highly diverse in seaweeds, which makes exact predictions difficult. We hypothesized that the response of a wide range of seaweed morphologies to drag may be predicted with good approximation if they are regarded as ellipsoids. A set of simple shape descriptors (wet weight, volume, thallus thickness, thallus projection area) and a solution for the drag equation were identified that together allowed for a relatively accurate prediction of the sinking velocity of morphologically diverse specimens belonging to 13 species of SW Baltic macroalgae (Fucus vesiculosus, Fucus serratus, Chorda filum, Saccharina latissima, Ahnfeltia plicata, Ceramium sp., Furcellaria lumbricalis, Gracilaria vermiculophylla, Vertebrata fucoides, Chaetomorpha linum, Cladophora sp., Ulva compressa and Ulva intestinalis), as well as of eelgrass Zostera marina that is another major source of drifting biomass in the region. The drifting behavior of seaweeds in currents could also be predicted fairly well by the model, although ground friction reduced the accuracy to some extent in non-buoyant species. Waves reduced the drifting velocity of fast drifting and positively buoyant seaweeds in tendency more than that of slower drifting specimens.

### SYMPOSIUM 10 "Ecology of algal systems"

#### Declining recruitment in a potential climate refuge for kelp

#### Barrientos, S.\*, Piñeiro-Corbeira, C. & Barreiro, R.

BioCost Research Group, Facultad de Ciencias and Centro de Investigaciones Científicas Avanzadas (CICA), Universidad de A Coruña, 15071, A Coruña, Spain \*sara.barrientos@udc.es

Kelp forests are in decline in some portions of their distribution area due to several threats related to global change, including a recruitment failure. However, kelp recruitment failure did not seem to be a problem in degraded forest of Ría de Vigo, northwest Spain. Here, a previous seasonal study conducted during one year on healthy and degraded kelp (*Laminaria ochroleuca*) reefs found a high abundance of recruits during spring, but most of recruits on degraded reefs failed to become adults due to fish herbivory.

To test whether the absence of reproductive adults was causing a decline in recruitment, the abundance of recruits was estimated seasonally over three years in the same previously studied kelp reefs in northwest Spain. Generalized linear mixed models (GLMM) were used to test the effect of *Year*, *Season* 

and *Location* as fixed factors and *Site* as random effect on the abundance of kelp recruits. Results showed a decline in the abundance of recruits in the third year compared to the first one for most of the seasons regardless of location and conservation status of the reef. However, a detailed analysis of spring data, the season of greatest decline in recruit abundance throughout the study after an initial peak, did show significant differences according to reef location.

This drop in recruitment suggests that degraded reefs of Ría de Vigo may disappear completely over time, and furthermore, this threat appears to be spreading to other apparently healthy reefs in this potential kelp climate refuge.

### Site-specific predictive models for estimating wet biomass of macroalga *Gongolaria barbata* from two sites

Bilajac, A.\*, Gljušćić, E., Smith, S. & Iveša, L.

Laboratory for the ecology of benthos, Ruđer Bošković Institute, Center for Marine Research Rovinj - Croatia \*abilajac@irb.hr

*Gongolaria barbata* is an important canopy-forming macroalga providing critical ecosystem services in intertidal and subtidal zones of the Mediterranean Sea. However, in recent years its populations along the Istrian coast (northern Adriatic Sea) have sharply declined. Therefore, development and application of non-destructive methods for long-term monitoring of remnant populations is necessary. This study aimed to generate site-specific predictive models for estimating wet biomass using morphometric variables that can easily be measured in the field. Morphometric characteristics, such as total cauloid length, cauloid length, number of cauloids, and wet biomass, were measured on 32 individuals from rockpools and 70 individuals from a lagoon. Linear uni- and multivariate models were created for each study population, and the best models were selected based on the corrected Akaike information criterion. Furthermore, a resampling experiment with replacement was conducted using the lagoon dataset to determine the minimum sample size required for creating site-specific predictive models.

The univariate model using the total cauloid length variable was the best predictive model for both populations. The model for the lagoon exhibited a strong fit, with an R-squared value of 0.88, while the model for the rockpools had a lower R-squared value of 0.51. A reliable model could be created using a minimum sample size of 40 or more individuals. In conclusion, the predictive models developed in this study using the total cauloid length variable could be useful for future monitoring of *G. barbata* populations, but in a site-specific context.

### *Lithophyllum byssoides* rims: the first marine Mediterranean ecosystem threatened of collapse?

Blanfuné, A.<sup>1\*</sup>, Boudouresque, C.F.<sup>1</sup>, Verlaque, M.<sup>1</sup>, Minne, A.<sup>2</sup>, Noisette, F.<sup>3</sup> & Thibaut, T.<sup>1</sup>

<sup>1</sup>Aix Marseille University, Université de Toulon, CNRS, IRD, MIO (Mediterranean Institute of Oceanography), UM 110, 13288, Marseille, France

<sup>2</sup>Oceans Institute and School of Biological Sciences, University of Western Australia, Crawley, WA, Australia <sup>3</sup>Institut des Sciences de la Mer, Université du Québec à Rimouski, Rimouski, QC G5L 3A1, Canada \*aurelie.blanfune-thibaut@mio.osupytheas.fr

The calcified Rhodophyta *Lithophyllum byssoides*, a very common midlittoral species in the western Mediterranean Sea, is a significant ecosystem engineer capable, under exposed and dim light conditions,

of building wide and solid endemic bioconstructions near the mean sea level: the *L. byssoides* rims or '*trottoirs à L. byssoides*'. Although the growth of the species is relatively rapid for a calcified alga, the construction of a large rim requires several centuries of near stable or slowly rising sea level. The time scale of their formation being measured in centuries, *L. byssoides* bioconstructions constitute valuable and sensitive sea level markers. The health status of *L. byssoides* rims has been studied at two sites located far apart from each other (Marseille and Corsica), both in heavily and few protected areas (Marine Protected Areas and unprotected areas). A health index: *L. byssoides* Rims Health index (LBRHI) is proposed. The health of *L. byssoides* rims in the two study areas is poor, with a LBRHI index ranging between 0.11 and 0.69 in Marseille (mean =  $0.34 \pm 0.07$ , SD = 0.20) and only between 0.03 and 0.32 in Corsica (mean =  $0.18 \pm 0.03$ , SD = 0.10). The major and unavoidable threat is the global rise in sea level. This ecosystem would be worldwide the first case of marine ecosystem collapse resulting, indirectly, from the human-induced global change.

#### Safety considerations of seaweed in Senegal: heavy metals hazards

Bousso, N.C.<sup>1,2,3\*</sup>, Brehmer, P.<sup>3</sup>, Kane, C.<sup>1</sup>, Faye, M.<sup>1</sup>, Aroui Boukbida, H.<sup>4</sup>, Diedhiou, F.<sup>2</sup>, Diadhiou, H.D.<sup>5</sup>, Quack, B.<sup>6</sup> & Ndiaye, W.<sup>2</sup>

<sup>1</sup>Ecole Supérieure Polytechnique (ESP), UCAD, BP: 5085, Dakar, Sénégal

<sup>2</sup>ISRA, Centre de Recherches Océanographiques de Dakar Thiaroye, CRODT, Dakar, Sénégal

<sup>3</sup>IRD, Univ Brest, CNRS, Ifremer, Lemar, SRFC, Dakar, Sénégal

<sup>4</sup>Instrumentation, Moyens Analytiques, Observatoires en Géophysique et Océanographie, UAR IMAGO, IRD BP: 1386 Route des Hydrocarbures, Dakar, Sénégal

<sup>5</sup>Consultant en Pêche et Aquaculture, Freelance, Sipres 5, villa n 21, Cap des Biches, Dakar

<sup>6</sup>GEOMAR Helmholtz-Zentrum für Ozeanforschung Kiel (GEOMAR), 24105 Kiel, Germany

\*boussocoumba@yahoo.fr

To meet the problem of nourishing a global population of estimated 9.7 billion by 2050, as well as the impact of climate change on food production and use, expanded use of seaweed has been proposed. It is a promising approach for long-term development in West Africa. However, dangerous heavy metals such as arsenic (As), mercury (Hg), lead (Pb), and cadmium (Cd) can accumulate in seaweed and become toxic above a specific threshold. From April to July 2022, 41 samples of 25 different seaweed species were gathered along the Senegalese coast between the Cap-Vert peninsula and the Petite Côte. Microwave Plasma Atomic Emission Spectroscopy and XRF-fluorescence spectrophotometrie were used to determine concentrations. The results demonstrate that 36% of the studied specimens have As concentrations exceeding the Norwegian National Institute of Nutrition and Seafood Research (NIFES, 2016) threshold of 10 mg kg<sup>-1</sup> of dry seaweed. All specimens have Cd concentrations exceeding 0.5 mg kg<sup>-1</sup> of dry seaweed, a threshold for toxicity by French health agencies. For 97% of the samples, the values for Cd were less than 10 mg kg<sup>-1</sup>, which is the minimum concentration that can be determined with acceptable uncertainty. Hypnea musciformis, stranding in substantial quantities along Senegal's coastline, contained Pb concentrations higher than four times the maximum suggested limit by French health agencies. An extension of the study area to the entire coastline over two years was intended to identify seasonal variations in heavy metal concentrations.

# The unusual massive beaching of Sargassum around Dakar Peninsula (Senegal, Africa) in autumn 2022: opportunity or threat

Bousso, N.C.<sup>1,2,3\*</sup>, Brehmer, P.<sup>3</sup>, Stiger-Pouvreau, V.<sup>4</sup>, Gautier, M.<sup>4</sup>, Kane, C.<sup>1</sup>, Diop, M.S.<sup>2,3,5,6</sup>, Faye, M.<sup>1</sup>, Faye, S.<sup>2</sup>, Balde, B.S.<sup>2</sup>, Sonko, A.<sup>3</sup>, Diedhiou, F.<sup>2</sup>, Quack, B.<sup>6</sup>, Diadhiou, H.D.<sup>7</sup>, Fricke, A.<sup>8</sup>, Aroui Boukbida, H.<sup>9</sup>, Weinberger, F.<sup>6</sup> & Ndiaye, W.<sup>2</sup>

<sup>1</sup>Ecole Supérieure Polytechnique (ESP), UCAD, BP: 5085, Dakar, Sénégal

<sup>2</sup>ISRA, Centre de Recherches Océanographiques de Dakar Thiaroye, CRODT, Dakar, Sénégal

<sup>3</sup>IRD, Univ Brest, CNRS, Ifremer, Lemar, SRFC, Dakar, Sénégal

<sup>4</sup>Univ Brest, CNRS, IRD, Ifremer, LEMAR, F-29280 Plouzané, France

<sup>5</sup>Laboratoire de Botanique et Biodiversité (LBB), Département de Biologie Végétale, UCAD, BP: 5005, Dakar-Fann, Sénégal

<sup>6</sup>GEOMAR Helmholtz-Zentrum für Ozeanforschung Kiel (GEOMAR), 24105 Kiel, Germany

<sup>7</sup>Consultant en Pêche et Aquaculture, Freelance, Sipres 5, villa n 21, Cap des Biches, Dakar

<sup>8</sup>Leibniz-Institut für Gemüse- und Zierpflanzenbau (IGZ), Theodor-Echtermeyer-Weg 1 14979 Großbeeren, Germany

<sup>9</sup>Instrumentation, Moyens Analytiques, Observatoires en Géophysique et Océanographie, UAR IMAGO, IRD BP: 1386 Route des Hydrocarbures, Dakar, Sénégal

\*boussocoumba@yahoo.fr

Huge strandings of holopelagic Sargassum along the Senegalese coast were observed throughout the summer of 2022. The unusual nature of such stranding has mobilized the community, sparking questions from civic society and industrialists. We report on this Sargassum arrival to participate in a global scientific endeavour and to inform local stakeholders. Volunteers reported stranding from north to south of Senegal, harming the coastal life, including turtle fatalities. We analyzed the stranded biomass for taxonomic identity and heavy metal concentrations (As, Cd, Pb and Hg). We differentiated the Sargassum collected into six morphotypes based on morphological details. We noticed the presence of the species S. natans I, S. fluitans III and S. natans VIII. Chemical nuclear magnetic resonance (HR-MAS NMR) fingerprintings were obtained to compare morphotypes and sampling sites. In comparison to previous studies throughout the tropical Atlantic Ocean, preliminary analyses revealed low quantities of arsenic and high concentrations of cadmium and mercury in the thalli. For all these heavy metals except lead, the concentrations are higher in S. natans I. Arsenic concentrations were variable between sampling sites but lead and mercury concentrations were spatially homogeneous. For agricultural purposes, the maximum values for As and Cd were above some recommended limits. For animal feed, all As concentrations were below the permissible level (40 ppm EU, European Union). Additional analyses are underway to validate these first results and study the biochemical composition of the samples.

## Holopelagic *Sargassum* along a degradation gradient: variation in biochemical composition and impact on potential valorization

Châtelain, B.<sup>1\*</sup>, Stiger-Pouvreau, V.<sup>1</sup>, Bonesteve, A.<sup>1</sup>, Gloaguen, N.<sup>1</sup>, Thouard, E.<sup>2</sup>, Dromard, C.<sup>3</sup> & Connan, S.<sup>1</sup>

<sup>1</sup>Univ Brest, CNRS, IRD, Ifremer, LEMAR, F-29280 Plouzane, France <sup>2</sup>IFREMER, Unité Biodiversité et Environnement de la Martinique, 97231 Le Robert, France <sup>3</sup>BOREA, Université des Antilles, MNHN, Sorbonne Université, Université de Caen Normandie, CNRS, IRD, 97157 Pointe-à-Pitre, Guadeloupe, France \*Benjamin.chatelain@univ-brest.fr

Holopelagic *Sargassum* species are brown seaweeds forming drifting rafts in the tropical Atlantic Ocean and since 2011, responsible for massive stranding on Caribbean, Mexican and African coasts, with significant ecological, economic and health problems. This research is part of the ANR SAVE-C project, looking at the ecology, physiology and recovery of *Sargassum* biomass from rafts. Only three morphotypes have been observed in those rafts: *Sargassum natans* I, *S. natans* VII and *S. fluitans* III. Samples have been collected during two field campaigns in dry and wet seasons in 2021 on two islands in the French Antilles at three stations: nearshore, in the bays and stranded. It appears that their biochemical composition is completely different according to the island and the season. Moreover, these morphotypes differed in their composition when the rafts get closer to the coast or once stranded. Indeed, the physiology and the metabolome showed a different response according to the location of the raft on a near-shore/stranding gradient especially for the amino acid contents and according to the morphotype, in particular for the pigment composition, the protein compartment and the phenolic compounds.

These different biochemical compositions of the algae allow us to study the kinetics of degradation of the metabolome of each morphotype when stranded and also allow us to suggest the use of the *Sargassum* drifting or stranded depending on the valorization sector in order to not let this huge biomass decompose unnecessarily.

### Temporal fluctuation of metallic and metalloid concentrations in three morphotypes of floating holopelagic *Sargassum* from Caribbean coast (Guadeloupe, French West Indies)

Cipolloni, O-A.<sup>1</sup>\*, Couture, P.<sup>2</sup> & Pascal, P-Y.<sup>1</sup>

<sup>1</sup>Université des Antilles, Équipe Biologie de la mangrove, Institut de Systématique, Évolution, Biodiversité, ISYEB, UMR 7205, UFR SEN, 97100 Pointe-à-Pitre, France <sup>2</sup>L- Centre Eau Terre Environnement, INRS-ETE, Québec, Canada \*oceanne.cipolloni@gmail.com

Since 2011, the Caribbean coasts have experienced unprecedented stranding of a pelagic brown macroalgae *Sargassum* inducing damages for coastal ecosystems and economy. This study evaluated the temporal fluctuations of metallic trace elements (MTE) in *Sargassum* freshly arrived in Caribbean coast. From May 2020 to September 2021, 12 floating *Sargassum* samples of three morphotypes (*S. fluitans* III and *S. natans* I and VIII) were regularly and manually collected in the Petit Cul-de-Sac Marin (Guadeloupe, French West Indies). Measured concentrations of 28 metal(loid)s trace elements reveal *i*) an absence of MTE seasonality, except for the Al and the Fe more abundant during 2020 summer (June to August) *ii*) a regular and high As content during the entire survey *iii*) a similar trend of contamination for each morphotype. The constant and high amount of As imply that stranding management policy and valorization processes of *Sargassum* must consider As contaminant and that this vigilance must be constant along the year.

# Invasive alga *Rugulopterix okamurae* relies on high resilience to elevated CO<sub>2</sub> and nutrient limitation to expand in the Mediterranean

Cobos, P.\*, Iñiguez, C. & Gordillo F.J.L.

*University of Málaga, Spain* \*pablocobosmateo@uma.es

Invasive species, eutrophication and climate change are relevant problems for the survival of numerous ecosystems. In this work, the ecophisiological response of the brown alga species (Ochrophyta) *Rugulopteryx okamurae* has been studied towards some of the main global change drivers, considering 8 treatments as the result of the combination of two levels of  $CO_2$  (400 y 1000 ppm), two levels of nutrients (330 y 30  $\mu$ M de  $NO_3^-$  y 30 y 3  $\mu$ M de  $PO_4^{3-}$ ) and two different temperatures (15 °C and 23 °C). It has also been discussed the adjustment of rapid light curves (RLC). Growth rate has been fundamentally affected by temperature, whereas pigmentary and rapid light curves changes have been driven by interactions among all factors. Nutrient's incorporation rates measured in the last four days of the experiment have reported a relatively high nutrient uptake in this species. Interaction of the three factors regarding growth rate, phosphate (PO4<sup>3-</sup>) uptake suggests the capacity of this species of developing diverse acclimation modifications. Results show that this species development is viable under all conditions tested, being its vegetative growth enhanced by temperature, showing *Rugulopteryx okamurae* high plasticity, suggesting its huge invasive potential. Adding this to climate change, this species propagation may become greater over the years.

### Remote sensing vs. *in situ* data: a comparative analysis of seaweed cover assessment on rocky shores

Diruit, W.<sup>1</sup>\*, Barda-Chatain, M.<sup>2</sup>, Helias, M.<sup>1</sup>, Terrin, S.<sup>1</sup>, Burel, T.<sup>1</sup>, Bajjouk, T.<sup>3</sup>, Le Bris, A.<sup>4</sup>, Richier, S.<sup>4</sup> & Ar Gall, E.<sup>4†</sup>

<sup>1</sup>Univ Brest, CNRS, IRD, Ifremer, LEMAR, 29280 Plouzané, Brittany, France <sup>2</sup>Université de Pau et des Pays de l'Adour, Campus d'Anglet (Montaury), 64600 Anglet, France <sup>3</sup>Ifremer, DYNECO/LEBCO, 29280 Plouzané, Brittany, France <sup>4</sup>Centre d'Etude et de Valorisation des Algues (CEVA), 22610 Pleubian, Brittany, France \*Wendy.Diruit@univ-brest.fr

The diverse and dynamic communities of temperate rocky shores, structured by canopy forming Phaeophyceae, are under threat from anthropogenic and climate change driven pressures. To assess the health of these communities, remote sensing has become a popular tool in addition to field work. This study aims at comparing *in situ* data to vegetation indices defined through three different remote imagery approaches, with varying resolutions: satellite (50 cm), plane (30 cm), and UAV (2 cm). The study involved characterizing covers of dominant seaweeds at 24 sampling spots (2.7 m<sup>2</sup> each) on a single site using both field and remote sensing methods. The sampling spots were distributed vertically across four bathymetric levels that were dominated by Phaeophyceae: 1- *Pelvetia canaliculata* and *Fucus spiralis* (high shore), 2- *Ascophyllum nodosum* (mid shore), 3- *Fucus serratus* (low shore) and 4- *Himanthalia elongata* and *Laminaria digitata* (sublittoral fringe). Although the high shore *in situ* covers were significantly different from both vegetation indices, the subsequent bathymetric levels showed similar results regardless of the method or resolution used. Both vegetation indices provided consistent results for macroalgal-dominated habitats, changing predictably with the bathymetric level. While low resolution may suffice for estimating seaweed covers, high resolution is necessary for comprehensive monitoring of macroalgal communities.

## Matter of traits: Combined effects of nutrients and climate change on cyanobacterial bloom toxicity across scales

Drost, A.M.\*<sup>1,2</sup>, Huisman, J.<sup>2</sup> & Van de Waal, D.B.<sup>1,2</sup>

<sup>1</sup>Department of Aquatic Ecology, NIOO-KNAW, the Netherlands

<sup>2</sup>Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, the Netherlands \*a.drost@nioo.knaw.nl

Cyanobacterial blooms are on the rise due to climate change and eutrophication. These blooms can often be toxic and threaten water quality. The toxicity of these blooms depends not only on the biomass of cyanobacteria, but also on the type and amount of toxins produced by toxic species and genotypes. The effects of warming and eutrophication on blooms are well studied, but many questions remain about the complex interactions that shape cyanobacterial bloom toxicity, especially on scales beyond lab-studies. A potential mechanistic coupling between environmental factors and the toxins produced by cyanobacteria may be provided by ecological stoichiometry, which investigates how ecological interactions are shaped by the relative availability of energy (as carbon) and nutrients. Recent studies have furthermore shown that integrating ecological stoichiometry and trait-based ecology may allow scaling up from cellular traits to cyanobacterial bloom dynamics.

Here, we assess the temporal dynamics of cyanobacterial blooms, aiming to link cyanobacterial traits and the elemental composition to bottom-up and top-down processes to understand bloom toxicity. Specifically, we study the variation in carbon, nitrogen and phosphorus availabilities and ratios, together with abiotic factors such as temperature, and the association with higher trophic levels like grazing and parasites, to understand shifts in cyanobacterial bloom biomass, community dynamics, and toxicity.

These explicit links between cyanobacterial blooms, the prevailing traits, and both the abiotic and biotic environment will help to understand how global change may affect their toxicity, which in turn may inform future monitoring strategies for risks associated to harmful cyanobacterial blooms.

# Growth preferences of the haptophyte *Chrysochromulina leadbeateri* forming ichthyotoxic blooms in fjords in N Norway

Fon, M.<sup>1,2</sup>, Reitzel, H.<sup>2</sup>, Supraha, L.<sup>2,3</sup>, Andersen<sup>2</sup>, T., Eikrem<sup>2,3</sup>, W., Uhlig, S.<sup>1</sup> & Edvardsen, B.<sup>2\*</sup>

<sup>1</sup>Norwegian Veterinary Institute, Norway.
<sup>2</sup>University of Oslo, Department of Biosciences, Norway.
<sup>3</sup>Norwegian Institute for Water Research, Norway.
\*bente.edvardsen@ibv.uio.no

Microalgal flagellates of the genera *Chrysochromulina* and *Prymnesium* may form extensive blooms in marine and brackish waters causing fish kills. *Chrysochromulina leadbeateri* blooms caused massive fish kills in fish farms in Northern Norway in May 1991 and 2019. More than 14 500 tons of farmed salmon died in 2019. Cultures from both blooms were used in physiological experiments to investigate possible causes for the blooms. Growth experiments with varying salinity, temperature and irradiance in a facultative design were performed. Highest growth rates were obtained at temperatures 11-15 °C and salinities 25-30, and the tolerance range for growth were between 4-15°C and salinities 20-34. Under optimal conditions, growth rates increased with irradiance (up to 100 µmol photons  $m^{-2} s^{-1}$ ). During the bloom in 2019, highest *C. leadbeateri* concentrations were found in waters with slightly reduced salinity (32-33) and sea surface temperatures around 7°C. Strains from the blooms in 1991 and 2019 were

identical in the 18S rRNA gene. This ribotype appears to have a cold waterArctic geographical distribution based on the metaPR2 metabarcoding datasets, whereas other ribotypes of *C. leadbeateri* in this dataset show a cosmopolitan or temperate-warm water distribution. We suggest that *C. leadbeateri* consists of several ribotypes with different growth preferences and that the toxic blooms in Northern Norway were formed by a ribotype adapted to cold waters with slightly reduced salinity and can tolerate rather high irradiances. Possible causes for the ichthyotoxic blooms will be discussed.

#### Impacts of habitat fragmentation on macroalgal forests

Galobart, C.<sup>1</sup>\*, de Caralt, S.<sup>2</sup>, Vergés, A.<sup>2</sup>, Martín, D.<sup>1</sup>, Santamaría, J.<sup>1</sup>, Boada, J.<sup>1</sup>, Sitjà, C.<sup>1</sup> & Cebrian, E.<sup>1</sup>

<sup>1</sup>Centre d'Estudis Avançats de Blanes (CEAB-CSIC), Blanes, Spain <sup>2</sup>Universitat de Girona (UdG), Girona, Spain cgalobart@ceab.csic.es

Habitat loss resulting from anthropogenic activities is considered a major threat to biodiversity and ecosystem functioning. However, habitat fragmentation, the process of breaking up larger areas into smaller pieces, may occur much before complete habitat loss. Despite being scarcely studied in marine ecosystems, the fragmentation process is known to first have impacts on the primary and structural species of the habitat, followed by impacts on the associated assemblages, and further consequences to the stability of the ecosystem. Here, we aim to resolve how habitat fragmentation affects Mediterranean macroalgal forests dominated by the canopy-forming species Ericaria brachycarpa. To achieve this, we selected two different areas, the Catalan Coast and Balearic Islands. At each area, we sampled three different localities embracing sites with three fragmentation levels (high, medium and low). Our preliminary analyses showed that, in all localities, the biomass and density of the structural species decreased with habitat fragmentation. Furthermore, mature populations were absent in highly fragmented sites, while present in low fragmented ones. The consequences of the loss of density and biomass of the structural species were also evident in the associated community, especially on macroalgae, but also on mobile macroinvertebrate assemblages. Our study highlights the need of preserving well-conserved, continuous and structured Mediterranean macroalgal forests since even initial stages of degradation can significantly lead to the loss of biodiversity and key ecosystem functions.

## Epiphyte community in two prominent fucalean algae on the western Istrian coast

Gljušćić, E.\*, Bilajac, A., Maree Smith, S. & Iveša, L.

Laboratory for the ecology of benthos, Ruđer Bošković Institute, Center for Marine Research Rovinj - Croatia \*egljusc@irb.hr

Until the recent past, western Istrian coast (northern Adriatic) hosted rich fucalean assemblages. However, this has changed following a massive regression of *Cystoseira sensu lato* and *Sargassum sp.* marine forests which was documented with its peaks and ebbs since the late 1960's. Fucalean species can host complex communities in their canopies and potentially provide a plethora of ecosystem services such as feeding grounds, shelter, rearing areas and refugia from predation. Even though fucalean algae themselves are valuable, the epiphytes that can form a major part of the canopy are often being left out. To gain insight into epibiont community structure, two long living algae, *Gongolaria barbata* and *Ericaria crinita*, prominent fucalean species commonly hosting rich epibiota, were sampled in 5 different geographical areas to analyze their epiphytes according to host species, sampling locations and morpho-functional groups.

Statistical analysis shows that there is no difference in epiphyte species diversity or frequency between the host algae. However, areas do show some distinctiveness, particularly the southernmost site at cape Kamenjak. Further statistical analysis of the epiphytes revealed no difference between geographic areas or host species, although some site-specific species distribution and strong group dominance suggests that environmental factors play a role in determining epibiont diversity and distribution. Almost no "fucales-on-fucales" epiphytism was detected, which was often observed up until the recent past. The findings of this study provide valuable insights into the ecological relationships between algae and their associated epibionts, which could be important for the conservation and management of marine ecosystems.

# The mechanism of competition between coexisting aquatic taxa on the example of *C. glomerata* (L) Kütz. and *L. minor* L. and cyanobacteria in the body of water

Gostyńska, J.\*, Messyasz, B.

Department of Hydrobiology, Faculty of Biology, Adam Mickiewicz University in Poznań, Poland \*julia.gostynska@amu.edu.pl

The consequence of the large amount of nutrients in eutrophic waters in the excessive development of biological life, including green algae (*C. glomerata* (L) Kütz.) and pleustophytes (*L. minor* L.). The ecological balance is also distributed by the presence and intensive bloom of phytoplankton. The coexistence of several taxa in the same place and time can cause many interactions. One of them is competition, that can result in mutual substation of species, exclusion of species or separation of organic compounds.

The aim of the research is to answer the following questions:

(1) Do L. minor and C. glomerata secrete allelopathic substances in coexistence?

(2) Does allelopathic potential occur in competitive interactions?

(3) Does the negative impact on the condition and the survival of species increase with the exposure time and concentration of a polyphenolic acid?

Experiments were carried out using polyphenols secreted by *C. glomerata*: gallic acid, benzoic acid, quinic acid, coumaric acid at concentrations of 0.01mg/L; 0.10mg/L; 0.50mg/L; 1.00mg/L; 2.00mg/L; 5.00mg/L. The exposure time of polyphenols to L.minor and cyanobacteria was: 2 days, 7 days, 14 days. The results show that polyphenols produced by *C. glomerata* have a significant impact on the condition and survival of the species. The concentration of polyphenols in the cells of algae, pleustophytes and cyanobacteria increases with time, which causes a faster reaction of organisms. However, *C. glomerata* biomass grows faster than *L. minor* biomass. These analyzes confirm that allelopathy can be the result of competition and has a significant impact on shaping the structure of plant communities in the aquatic ecosystem.

### Sediments as reservoirs of unexpected pelagic diversity: Distribution of the dormancy trait in Baltic phytoplankton communities

Kremp, A.<sup>1\*</sup>, Savela, H., Lehtinen, S., Hällfors, H. & Suikkanen, S.

<sup>1</sup>*Institute for Baltic Sea Research Warnemuende, Germany* \*anke.kremp@io-warnemuende.de

In temporally variable environments, the formation of dormant resting stages is an effective bet-hedging strategy to secure short- and long-term fitness of populations and communities, and a buffer against environmental variability. Many phytoplankton species form dormant resting stages to survive unfavorable conditions. Pools of such resting stages form seed banks which integrate seasonal and annual cohorts of phylogenetically and physiologically different individuals. Describing the diversity and reservoir function of phytoplankton seed banks has been hampered by difficulties in identification of resting stages. While traditional approaches based on cyst- or hatched cell morphology is often limited by lack of distinctive features or complex germination conditions, DNA based identification of resting stages from sediments can be biased by cellular remains of settled dead cells or problems with DNA extraction.

We developed a sediment processing and DNA extraction protocol specifically targeting resistant phytoplankton resting stages in surface sediments, and analyzed community composition of seed banks in coastal and open sea areas of the Baltic Sea using metabarcoding. Results revealed an unexpectedly high number of taxa at the different sampling sites forming resistant resting stages, which by far exceeds previous estimates and includes many taxa previously not known to have a dormant phase in their life cycle. This suggests that the dormancy trait is much more prevalent in Baltic phytoplankton communities than previously thought. The implications of this finding for long term stability of phytoplankton communities will be discussed.

## Response of the cold-adapted spring bloom community to increased nitrogen in the Baltic Sea

Peter, C.<sup>1\*</sup>, Farnelid, H.<sup>1</sup>, Lindehoff, E.<sup>1</sup>, Mollica, T.<sup>1</sup> & Legrand, C.<sup>1,2</sup>

<sup>1</sup>Linnaeus University, Sweden <sup>2</sup>Halmstad University, Sweden \*carolin.peter@lnu.se

In the Baltic Sea, the magnitude and composition of the spring bloom are determined by both temperature and nutrient concentrations. While most studies focus on the dominating phyla, we assessed the response of different size classes to increased nutrient availability. A cold-adapted spring bloom community in the Baltic proper was incubated at 6  $^{\circ}$ C in four replicate mesocosms (100L) for 14 days. Nutrient additions (N-NO<sub>3</sub>, N-NH<sub>4</sub>, P) simulated either a singular eutrophication event or chronic eutrophication.

While communities were in all cases dominated by larger diatoms e.g *Skeletonema* spp., *Chaetoceros* spp, continuously high nutrient availability led to significantly shorter *Skeletonema* colonies and higher final cell concentrations. Within the small fraction ( $<20\mu$ m), the dominance shifted from pico- to nanoplankton, with the picocyanobacteria *Synechococcus* not benefitting from any of the eutrophication scenarios.

While nitrogen allocation in the large fraction (>50 $\mu$ m) showed great variability between treatments, the small fraction (<20 $\mu$ m) was less affected and increased in numbers even while facing nutrient depletion, potentially alluding to their mixotrophic character. When N-ammonium was supplied, a substantially bigger portion of carbon and nitrogen were allocated to the small fraction compared to N-nitrate addition, suggesting a significant role of nitrogen speciation in case of chronic eutrophication. Further data analysis will reveal the relative importance of autotrophs and heterotrophs. Our results

illustrate how chronic eutrophication and N-speciation alter the cold-adapted phytoplankton community dynamics, with a larger effect on the nutrient allocation in pico- and nanophytoplankton than previously shown for spring blooms in the Baltic Sea.

# *In vitro* effects of the harmful benthic dinoflagellates *Ostreopsis* cf. *ovata* and *Prorocentrum hoffmannianum* on immune responses of the farmed oyster *Crassostrea gasar*

Queiroga, F.R.<sup>1\*</sup>, Hegaret, H.<sup>1</sup>, Carvalho, W.F.<sup>2</sup>, Naveira, C.<sup>3</sup>, Rodrigues, N.<sup>3</sup>, Santos, F.S.<sup>3</sup>, Nascimento, S.M.<sup>3</sup> & Neves, R.A.F.<sup>3</sup>

<sup>1</sup>Laboratoire des Sciences de l'Environnement Marin, Institut Universitaire Européen de la Mer, France. <sup>2</sup>Department of Ecology and Marine Resources, Federal University of the State of Rio de Janeiro (UNIRIO), Rio de Janeiro, Brazil. <sup>3</sup>Research Group of Experimental and Applied Aquatic Ecology, Federal University of the State of Rio de

<sup>3</sup>Research Group of Experimental and Applied Aquatic Ecology, Federal University of the State of Rio de Janeiro (UNIRIO), Brazil.

\*fernando.ramosqueiroga@univ-brest.fr / queirofa\_fr@hotmail.com

Oyster production is an alternative to food production but it can be impacted by the presence of harmful microalgae like the dinoflagellates Prorocentrum hoffmannianum and Ostreopsis cf. ovata. This study aims to evaluate the in vitro effects of P. hoffmannianum and O. cf. ovata in immune system cells (hemocytes) of the oyster Crassostrea gasar. Firstly, the direct toxicity of both dinoflagellates was evaluated in eight concentrations under the hemocyte viability. No reduction in hemocyte viability was found with the exposure to cell culture or crude extract of P. hoffmannianum, but O. cf. ovata culture induced hemocyte death in a concentration-dependent manner. Ostreopsis cf. ovata concentration that promotes the half of maximal reduction in hemocyte viability ( $EC_{50}$ ) was 779 cells mL<sup>-1</sup>. Posteriorly, hemocytes were exposed to both dinoflagellate cell cultures and crude extracts to investigate the effects under functional parameters. The highest concentration used for P. hoffmannianum and three concentrations of O. cf. ovata (the lowest, the highest, and the equivalent to the  $EC_{50}$ ) were used. Prorocentrum hoffmannianum extract promoted a threefold increase in ROS production and a reduction of less than half of the phagocytosis rate. Ostreopsis cf. ovata cell cultures and crude extracts also promoted an increase in ROS production (two-fold), but the phagocytosis rate was reduced (half) only in response to the two lower concentrations of cell culture. These results indicate a noxious potential of both dinoflagellates through a direct toxicity and functional impairment of hemocytes which could expose C. gasar oyster to opportunistic infections.

## Warming rate shapes the thermal tolerance of freshwater phytoplankton

Sánchez de Pedro, R<sup>\*.1</sup>, Melero-Jiménez, I.J.<sup>1</sup>, Reul, A.<sup>2</sup>, Viruel, M.<sup>3</sup>, Bañares-España, E.<sup>1</sup>, Flores-Moya, A. & García-Sánchez, M.J.<sup>1</sup>

<sup>1</sup>Universidad de Málaga, Andalucía Tech, Departamento de Botánica y Fisiología Vegetal, Campus de Teatinos, 29010 Málaga, Spain

<sup>2</sup>Universidad de Málaga, Andalucía Tech, Departamento de Ecología y Geología, Campus de Teatinos, 29010 Málaga, Spain

<sup>3</sup>Instituto de Hortofruticultura Subtropical y Mediterránea La Mayora, UMA-CSIC, 29750, Algarrobo-Costa, Málaga, Spain

\* rsdpc@uma.es

The impact of global warming on phytoplankton species in freshwater ecosystems could vary depending on their capacity for adapting and acclimating to changes in temperature. To make better predictions under different global warming scenarios, it is crucial to perform long-term experiments where the selection of new genetic variants could occur in addition to potential gene expression changes.

Here we aimed to explore the differential evolutionary potential of two freshwater phytoplankton species growing under increasing temperatures, the bloom forming cyanobacterium *Microcystis aeruginosa* (Kützing) Kützing and the green microalga *Chlamydomonas reinhardtii* P. A. Dang. We performed an evolutionary ratchet experiment lasting over 60 generations to detect the limit of resistance to high temperatures of both species. Populations were initially maintained at 25 °C and then subjected to a slow (+2 °C) or to a rapid (+4 °C) temperature increase. To characterize the increase in thermal tolerance of the derived populations, we compared the growth rate and photosynthetic performance of derived and ancestral populations at control (25 °C), sublethal and lethal temperatures of ancestral populations.

We observed that slow warming facilitates the increase of thermal tolerance of both species. Specifically, the lethal temperature of ancestral populations was only surpassed by derived populations under slow warming conditions. Thermo-tolerant derived strains showed higher photosynthetic capacity than ancestral ones at high temperatures, but at the cost of a limited capacity to dissipate energy via non-photochemical pathways. Our results suggested that the rate of global warming could modulate the thermal tolerance limit and resilience of phytoplanktonic species and alter the primary production and resilience of freshwater ecosystems.

## Early steps in the primary succession of seaweed communities at the Tajogaite volcano lava flows (Canary Islands, Spain)

Sangil, C.\*, Álvarez-Canali, D., Reyes, J., Rodríguez, J., Liulea, S., Afonso-Carrillo, J., Concepción, Y., Rancel-Rodríguez, N. & Sansón, M.

Marine Botany Group, Department of Botany, Ecology and Plant Physiology, Canary Islands, Spain \*casangil@ull.edu.es

On September 19 of 2021 the Cumbre Vieja volcano ridge (La Palma Island) erupted. The lava flowed from 1100 m height to the sea generating several lava deltas and new and sterile rocky reefs. Two months after the eruption ended, we started to monitor these new habitats with the aim of describe the primary succession of the seaweed communities. Samplings were performed in the intertidal as well as in the subtidal from 5 to 20 m depth at 2, 4.5, 7, 9 and 13 months. In addition, the study design included a control zone, the lava deltas of the San Juan volcano (1949) to contrast the succession with stable-stage communities.

The total seaweed cover was high in the lava flows and similar to the control zone from the beginning of the monitoring. In both in the intertidal and in the subtidal habitats, the substrate was almost completely covered by algae. Richness and species composition of communities was different in the two studied habitats. The number species in lava flows was initially low but gradually increased after 2 months, however after 13 months many species in the control zone had not yet colonized the deltas.

Communities in the primary succession had similar structure in both habitats, and initially were dominated in terms of cover by fast-growing species such as filamentous brown algae and diatoms. Over time, perennial species have gradually been incorporated, but even after 13 months the communities in the deltas were still different from those in the control zone. However, in the intertidal the succession has been faster than in the subtidal due to the larger incorporation of perennial species, and therefore these communities have begun to resemble those of a climax community.

# Ecology and phylogeny of alien *Raphidiopsis raciborskii*, *Chrysosporum bergii*, *Sphaerospermopsis aphanizomenoides* and *Cuspidothrix issatchenkoi* from three hypertrophic Lithuanian lakes

Šuikaitė, I.<sup>1</sup>\*, Karosienė, J.<sup>1</sup>, Savadova-Ratkus, K.<sup>1</sup>, Burokienė, D.<sup>2</sup>, Čepukoit, D.<sup>2</sup> & Koreivienė, J.<sup>1</sup>

<sup>1</sup>Laboratory of Algology and Microbial Ecology, Nature Research Centre, Akademijos Str. 2, Vilnius, LT-08412, Lithuania

<sup>2</sup>Laboratory of Plant Pathology, Nature Research Centre, Akademijos Str. 2, Vilnius, LT-08412, Lithuania \*izabele.suikaite@gamtc.lt

In recent years, cyanobacterial invasions have increased in European temperate lakes. The interactions between invaders and local species affect the composition of the phytoplankton community. As a result, biodiversity, cyanotoxin content and ecological functioning are affected. The establishment of newly arrived species is directly related to fluctuating physicochemical factors such as light, temperature, and nutrient concentrations, especially phosphorus and nitrogen, or biotic factors such as competition. In this study, we present four alien cyanobacteria, Raphidiopsis raciborskii, Chrysosporum bergii, Sphaerospermopsis aphanizomenoides and Cuspidothrix issatchenkoi, in three hypertrophic Lithuanian lakes (Simnas, Jieznas and Gineitiškės), which are among the northernmost localities of these species in Europe. The species were identified based on morphological characteristics and phylogenetic analysis. This study assessed temporal variation in the cyanobacterial composition and biomass during summer to determine the local dominant species with which the alien species compete. The biomass of alien species had a significant positive correlation with temperature and total nitrogen concentration. Enzymelinked immunosorbent assay (ELISA) results indicated the presence of microcystins, anatoxins and saxitoxins in the field samples. Polymerase chain reaction (PCR) for cyrJ, cyrA and sxtA genes were negative in the alien species cultures, indicating that the alien species were not potential producers of cylindrospermopsin and saxitoxin in these lakes. This work describes the ecology of alien cyanobacterial species in the early stages of their establishment and contributes to the understanding of the driving forces of their spread in the northern localities.

### Grazing effects on recruits of Mediterranean canopy-forming macroalgae: implications for conservation actions

Verdura, J.<sup>1\*</sup>, Monserrat, M.<sup>1</sup>, Annachi, A.<sup>2</sup>, Bianchelli, S.<sup>3</sup>, Cebrian, E.<sup>4</sup>, Chiarore, A.<sup>5</sup>, Colletti, A.<sup>6</sup>, Comeau, S.<sup>7</sup>, Danovaro, R.<sup>3</sup>, Fabbrizzi, E.<sup>5,6</sup>, Fraschetti, S.<sup>6</sup>, Kefalas, G.<sup>8</sup>, Lo Martire, M.<sup>3</sup>, Martini, F.<sup>3</sup>, Munari, M.<sup>9</sup>, Nardiello, M.<sup>6</sup>, Nakou, K.<sup>8</sup>, Orfanidis, S.<sup>8</sup>, Papadimitriou, A.<sup>8</sup>, Nasto, I.<sup>10</sup>, Romero, G.<sup>1</sup>, Santamaría, J.<sup>4</sup>, Silvestrini, C.<sup>6</sup>, Sitjà, C.<sup>4</sup>, Tamburello, L.<sup>5</sup> & Mangialajo, L.<sup>1</sup>.

<sup>&</sup>lt;sup>1</sup>Université Côte d'Azur, CNRS, UMR 7035 ECOSEAS, Nice, France

<sup>&</sup>lt;sup>2</sup>University of Carthage, Bizerte, Tunisia

<sup>&</sup>lt;sup>3</sup>Polytechnic University of Marche, Italy

<sup>&</sup>lt;sup>4</sup>Centre d'Estudis Avançats de Blanes CEAB-CSIC, Blanes, Girona, Spain

<sup>&</sup>lt;sup>5</sup>Stazione Zoologica Anton Dohrn, Napoli, Italy

<sup>&</sup>lt;sup>6</sup>Università di Napoli Federico II, Napoli, Italy

<sup>&</sup>lt;sup>7</sup>Sorbonne Université, CNRS, Laboratoire d'Océanographie de Villefranche, Villefranchesur-Mer, France

<sup>8</sup>Fisheries Research Institute, ELGO-DIMITRA, Greece
 <sup>9</sup>Department of Biology, University of Padova, Padova DiBio-UNIPD, Italy
 <sup>10</sup>University of Vlora "Ismail Qemali", Vlora, Albania
 \*jana.verdura@univ-cotedazur.fr

Herbivory is known to be one of the major factors responsible for the decline of marine forests in temperate areas, driving regime shifts to turf-dominated communities or barren grounds. In the Mediterranean Sea, sea urchins and herbivorous fish have been considered the primary herbivore consumers of canopy-forming macroalgae. However, mesograzers may also threaten macroalgal forests, especially affecting their early-life stages. Herbivory on early-life stages can be one of the main factors hindering the success of macroalgal restoration. Yet, despite being an essential process of regulating population abundance and dynamics, understanding factors influencing recruitment is still limited. To better understand the role of herbivory on early-life stages of Cystoseira sensu lato species, we performed mesocosm experiments focussed on mesograzers complemented by in situ exclusion experiments in the French Riviera. Our results highlight the importance of herbivory on the survival of early-life stages. Additionally, the preliminary results of a collaborative study across the Mediterranean Sea, performed in the framework of the AFRIMED project, showed a high variability across areas. This suggests a context-dependency of processes, due to local factors directly affecting recruitment and potentially masking herbivory effects, such as the presence of a macroalgal forest. Both small-scale and regional studies are particularly relevant for guiding the spatial planning of conservation and restoration interventions on these important ecosystems.

## SYMPOSIUM 11 "Changing distribution patterns and new ecosystems"

#### Development of benthic diatoms on natural and artificial substrates in marine lake (South Adriatic Sea)

Car, A.<sup>1\*</sup>, Dupčić Radić, I.<sup>1</sup>, Hafner, D.<sup>2</sup>, Kaleli, A.<sup>3</sup>, Ljubimir, S.<sup>4</sup> & Nadir Solak, C.<sup>5</sup>

- <sup>1</sup>University of Dubrovnik, Institute for Marine and Coastal Research, 20000 Dubrovnik, Croatia <sup>2</sup>Bartulovici 4, 20357 Blace, Croatia
- <sup>3</sup>Department of Marine and Freshwater Resources Management, Faculty of Aquatic Sciences, Istanbul University, Istanbul, Turkey

<sup>4</sup>Matije Gupca 5, 20000 Dubrovnik, Croatia

<sup>5</sup>Department of Biology, Faculty of Science and Arts, Kütahya Dumplupinar University, Kütahya, Turkey \*ana.car@unidu.hr

The diatom fouling on a particular substrate is strongly influenced by the substrate properties and preferences of a diatom species. A distinction between substrates can be made by analyzing the specific abundance and composition of diatoms on different substrates. In this study, a comparison was made between an artificial glass substrate and two substrates that form a natural habitat (macroalgae and rocks).

Samples were collected weekly between 11 August and 2 September 2016 at a sampling site in a shallow marine lake, the Mrtvo More (Dead Sea) on Lokrum Island near Dubrovnik (South Adriatic, Croatia). In addition to the detailed light microscopic analysis, an ultrastructural analysis of the benthic diatoms from lake Mrtvo More was carried out for the first time using scanning electron microscopy (SEM). A total of 97 taxa were identified. *Cocconeis scutellum* Ehrenberg and *Halamphora coffeiformis* (C. Agardh) Mereschkowsky were the most frequent taxa in the samples. Shannon-Wiener diversity index (H') values varied from 1.78 (in September on Padina sp.) to 4.52 (in August on glass).

The differences in the number of diatoms colonizing the different substrates highlight the need for caution when selecting a substrate on which to study the colonization of organisms, especially if the experiments are to be used for subsequent predictions. The results of the analysis showed that the diatom communities developed on the artificial substrate accurately correspond to the diatom community of a rock substrate and can therefore be used in further experiments as a representative alternative for studies of epilithic diatoms.

#### Repeated evolution of asexuality in the brown alga Scytosiphon

Hoshino, M.<sup>1,2</sup>, Cossard, G.<sup>1</sup>, Haas, F.<sup>1</sup> & Coelho, S.M.<sup>1\*</sup>

<sup>1</sup>Department of Algal Development and Evolution, Max Planck Institute for Biology Tübingen, 72076 Tübingen, Germany.

<sup>2</sup>*Current address: Research Center for Inland Seas, Kobe University, Rokkodai 1-1, Nadaku, Kobe 657-8501, Japan.* 

\*susana.coelho@tuebingen.mpg.de

Sexual reproduction is widespread among eukaryotes, but asexual lineages have repeatedly evolved from sexual ancestors across a wide range of taxa. Despite extensive research on the evolution of sex, the molecular changes underpinning the switch to asexual reproduction remain elusive, particularly in organisms with haploid sexual systems such as bryophytes, red and brown algae. Here, we explore three independent events of emergence of asexuality from sexual ancestors in brown algal natural populations of Scytosiphon species complex to examine the nature, evolution and degree of convergence of gene expression changes that accompany the breakdown of sexuality. We show that asexual, female-only populations arose in cold water environments and consistently exhibit a decay of sexual traits, including loss of capacity to fuse with male gametes, rapid trigger of parthenogenic development from unfertilized eggs, larger gamete size and loss of pheromone production. The decay in sexual traits is accompanied by dramatic changes in gene expression patterns, and an overall defeminization and masculinization of asexual female gamete transcriptomes. We will show how these data is being used to test the importance of sexual conflict on gene expression changes following loss of sexual reproduction and to tackle the molecular genetic mechanisms underlying pheromone production in these organisms.

#### Influence of physico-chemical parameters on colonization of bacteria and diatoms in a marine lake in southern Adriatic (NE Mediterranean)

Dupčić Radić, I.<sup>1\*</sup>, Car, A.<sup>1</sup>, Hafner, D.<sup>2</sup>, Ljubimir, S.<sup>3</sup>, Bobanović-Ćolić, S.<sup>1</sup> & Jasprica, N.<sup>1</sup>

<sup>1</sup>University of Dubrovnik, Institute for Marine and Coastal Research, 20000 Dubrovnik, Croatia <sup>2</sup>Bartulovici 4, 20357 Blace, Croatia <sup>3</sup>Matije Gupca 5, 20000 Dubrovnik, Croatia

#### \*iris@unidu.hr

The influence of physico-chemical parameters on the initial colonization of bacteria and diatoms on a submerged artificial substrate and the development of diatom communities was studied weekly from April to October 2016 at a station in the marine lake Mrtvo More in southern Croatia. The physico-chemical parameters (temperature, salinity, oxygen saturation, Chl *a* concentrations, nutrients: silicate, phosphate, nitrate, nitrite, ammonium) varied significantly between months and seasons.

The highest values of phosphate, silicate and especially Chl *a* were recorded on 20<sup>th</sup> of July. According to the TRIX trophic index, the lake showed different trophic characteristics: (i) oligotrophic (at the beginning and end of the study), (ii) mesotrophic (end of June to midJuly), (iii) eutrophic (end of July to mid-September). The heterotrophic bacteria peaked (69,268 cells cm<sup>-2</sup>) in early June, when the diatoms begin to increase. The lake has a high species richness of diatoms (285 diatom taxa within 72 genera), with the highest species diversity index in August. Among the diatoms, adnate were the primary colonizers, especially *Cocconeis dirupta* W. Gregory var. *flexella* (Janisch and Rabenhorst) Grunow and *Cocconeis scutellum* Ehrenberg var. *scutellum*, while motile taxa joined the fouling communities from July to September.

This study showed that of the physico-chemical parameters, temperature, salinity and nitrate concentration have the greatest influence on the abundance of diatom species and that species diversity increases with the enrichment of nutrient concentration. Strong correlations were found between environmental variables and diatoms, and shifts in dominance at the species level were noted.

### Predicting thermal tolerance limits of marine forests' seaweed species under a changing climate

Hernández-Chan, S.<sup>1,2</sup>\*, Uribe, G.<sup>3</sup>, Michelsen, S.<sup>4</sup>, Cardoso, P.G.<sup>1</sup>, Lima, F.<sup>2,5</sup> & Arenas, F.<sup>1</sup>

<sup>1</sup>Interdisciplinary Centre for Marine and Environmental Research (CIIMAR), University of Porto, Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos s/n, 4450-208 Matosinhos, Porto, Portugal. <sup>2</sup>Instituto de Ciências Biomédicas Abel Salazar da Universidade do Porto (ICBAS), University of Porto, Rua Jorge de Viterbo Ferreira 228, 4050-313 Porto, Portugal.

<sup>3</sup>Facultad de Ciencias, Universidad de Granada, Av. De Fuente Nueva s/n, 18071 Granada, Spain.

<sup>4</sup>Uppsala university, Department of Geology and Earth Sciences, Villavägen 16, 75236 Uppsala, Sverige, Sweden.

<sup>5</sup>Centro de Investigação em Biodiversidade e Recursos Genéticos (BIOPOLIS–CIBIO), Rua Armando Quintas 7, 4485-661 Vairão, Porto, Portugal.

\*sofia.hernandez.chan@gmail.com / shernandez@ciimar.up.pt

Marine seaweed forest habitats, such as kelp forests, have experienced a global decline over the past few decades due to the adverse effects of climate change (CC), particularly due to extreme marine heatwaves (MHWs). Characterized by a period of excessive warmth above a specific threshold, MHWs are predicted to increase in intensity, duration and frequency in the near future. However, significant knowledge gaps in our understanding of MHWs' impacts on marine forests hinder the development of accurate predictions for their resilience and the implementation of effective conservation policies.

Thermal tolerance (TT) assessments with ectothermic organisms show there is an exponential relationship between tolerance time and temperature, where heat tolerance decreases with increased exposure time to a thermal challenge. To better understand TT of marine forests macroalgae species, we adapted Rezende's and Jorgensen's unifying TT limit frameworks and applied it for the first time in macroalgae. Our assessments involved dynamic ramping temperature experiments, where thermic stress

and critical thermal maxima were evaluated via chlorophyll fluorescence quantification, to determine specific thermal performance curves and estimate tolerance probability functions for each species. Our fitted estimates closely resemble empirical values obtained from the literature across different thermal regimes, allowing us to predict species' thermal limits and the intensity of selection under

changing temperature regimes and projected MHWs. Our findings have significant implications for the conservation and management of marine forest ecosystems, as this framework could help us predict species' thermal tolerance and anticipate their responses to CC.

#### Evaluation of short-term copper toxicity in Synechococcus sp, Chaetoceros gracilis and Pleurochrisys roscoffensis under changes of temperature and salinity

Kholssi, R.<sup>1,2</sup>\* & Moreno-Garrido, I.<sup>2</sup>

<sup>1</sup>Composting Research Group, Faculty of Sciences, University of Burgos, Burgos, Spain <sup>2</sup>Institute of Marine Sciences of Andalusia (ICMAN-CSIC), Campus Río San Pedro, 11510 Puerto Real, Cádiz, Spain \*rajae.kholsi@gmail.com

Heavy metals such as copper (Cu) enter to marine environment through various sources causing changes in the biodiversity of marine microalgae. However, this metal plays a dual role as either micronutrient or toxicant (when supplied en excess). Researchers have suggested that copper becomes more toxic to microalgae species by alterations in environmental factors (due, for instance, to climate change). The purpose of this study was to assess the toxicity of Cu at low total concentration  $C1=2 \mu g L^{-1}$  and C2=5 $\mu$ g L<sup>-1</sup> in multispecies bioassays of three common species from phytoplankton (one cyanobacteria, Synechococcus sp., and two microalgae, Chaetoceros gracilis and Pleurochrisys roscoffensis) under combination of two levels of temperature (20 and 23 °C), and two levels of salinity (33 and 36) in a 96 h study using flow cytometry analysis. Generation of reactive oxygen species (ROS) of the mixed cultures by 2'7'-dichlorofluorescein (DCFH) was also measured by flow cytometry. Results indicated that Synechococcus sp. was more sensitive than Chaetoceros gracilis and Pleurochrisys roscoffensis to Cu stress at temperature 23°C and salinity of 36 under both concentrations. In addition, ROS levels at 23°C 33 and 5  $\mu$ g L<sup>-1</sup> of Cu were higher than all others combination studied. Since the test organisms represent important groups for marine ecosystems functions, our findings may raise some concerns on the impacts caused by possible future climate change scenarios in aquatic habitats chronically exposed to the frequent or intensive use of Cu.

# Assumptions and model structure for the growth and survival of the holopelagic brown macroalga *Sargassum* spp. based on DEB (Dynamic Energy Budget) theory

Lagunes, M-J.\*<sup>1</sup>, Pecquerie, L.<sup>2</sup>, Benavides, M.<sup>1</sup>, Cecchi, P.<sup>3</sup>, Changeux, T.<sup>1</sup>, Connan, S.<sup>2</sup>, Duarte-Neto, P.<sup>4</sup>, Jouanno, J.<sup>5</sup>, Klein, J.<sup>3</sup>, Lavaud, R.<sup>6</sup>, Le Loc'h, F.<sup>2</sup>, Lett, C.<sup>3</sup>, Lucena Frédou, F.<sup>7</sup>, Michotey, V.<sup>1</sup>, Munaron, J-M.<sup>2</sup>, Freile-Pelegrín, Y.<sup>8</sup>, Poggiale, J-C.<sup>1</sup>, Robledo, D.<sup>8</sup>, Stiger-Pouvreau, V.<sup>2</sup>, Stosic, B.<sup>4</sup>, Thibaut, T.<sup>1</sup>, Vásquez-Elizondo, R.M.<sup>8</sup> & Berline, L.<sup>1</sup>

<sup>1</sup>*Aix Marseille Univ, Université de Toulon, CNRS, IRD, MIO, Marseille, France* <sup>2</sup>*LEMAR, Univ Brest, CNRS, IRD, Ifremer, IUEM, F-29280 Plouzané, France* 

#### POSTERS

<sup>3</sup>MARBEC, Univ. Montpellier, CNRS, Ifremer, IRD, Montpellier, France
 <sup>4</sup>DEINFO, Univ. Federal Rural de Pernambuco, Recife, Brazil
 <sup>5</sup>LEGOS, Université de Toulouse, CNES/CNRS/IRD/UPS, Toulouse, France
 <sup>6</sup>School of Renewable Natural Resources, Louisiana State University Agricultural Center, Baton Rouge, LA 70803, USA.
 <sup>6</sup>DEPAQ, Univ. Federal Rural de Pernambuco, Recife, Brazil
 <sup>8</sup>CINVESTAV, Applied Phycology Lab, Department of Marine Resources, Mérida Unit, Mérida, México
 \*maria-jose.lagunes@mio.osupytheas.fr

Understanding and forecasting the proliferation of holopelagic *Sargassum* in the tropical Atlantic Ocean requires knowledge on the biology and transport of these macroalgae. Forecasting the biomass, proliferation and distribution of *Sargassum* rafts at time scales greater than a few weeks needs improved modeling. Recent modeling efforts focused mostly on transport and less on the biology of *Sargassum*. Three morphotypes in varying proportions compose these *Sargassum* rafts. Better understanding and modeling the specificities of each of the three morphotypes is the current challenge.

The main objectives of the BIOMAS project (BIOenergetic Modeling Approach for *Sargassum* dynamics) are to acquire the necessary knowledge to build an individual morphotype based model of *Sargassum* growth, to include this model into a drift model, and finally perform simulations of the integrated drift-growth model in order to forecast *Sargassum* morphotypes proliferation at seasonal scale. A strong synergy between laboratory experiments and modeling will allow us to build the first DEB (Dynamic Energy Budget) model applied to *Sargassum* and apply it to the three morphotypes. *Insitu* monitoring of *Sargassum* morphotypes in the Western and Eastern Atlantic will allow us to validate the integrated model.

We here focus on the description of the assumptions and structure of the DEB model for *Sargassum* growth and survival. Specific assumptions to differentiate each morphotype are discussed and model simulation will be used to contribute to the design of optimal protocols for experiments so that the parameters are accurately estimated.

### Characterisation of opportunistic bloom-forming macroalgae other than *Ulva* sp. in green tide bays of Brittany

Lasbleiz, M.\* & Richier, S.

*Centre d'Etude et de Valorisation des algues, France* \*Marine.lasbleiz@ceva.fr

The seasonal proliferations of green seaweed under the morphological blade-type *Ulva* have been observed on Brittany coasts since the late 60s-early 70s. These so-called green tides are directly related to the eutrophication of coastal waters, and especially to high terrigenous inputs of nitrogen to the sea. However, since 2006, massive proliferations of new opportunistic macroalgae have been recorded in several Brittany bays. These new bloomforming macroalgae locally or temporarily replace the green tide algae *Ulva*.

This study aims to understand the causes of such changes in bloom-forming macroalgal communities in Brittany bays, and questions the link between the occurrence of these new opportunistic species with the global improvement in water quality (associated with the decrease in nitrogen inputs coming from the land). Laboratory experiments were performed to characterize species-specific physiological traits (growth rates, nutrient uptake kinetic for nitrate, ammonium and phosphate) of four new bloom-forming macroalgae (the green macroalgae belonging to the genus *Ulvaria* and *Cladophora*, the red filamentous *Polysiphonia* sp., and the brown filamentous *Ectocarpus* sp.) in comparison with the reference green

tide algae *Ulva*. The results show that (1) *Cladophora* sp. and *Ectocarpus* sp. are more competitive than *Ulva* for nitrate uptake at low nitrate levels, explaining why they develop while *Ulva* declines in summer; and that (2) other environmental factors (*e.g.* winter algal stocks, seawater temperature, light) might be involved in changes in bloom-forming macroalgal communities in Brittany bays, the impact of climate change not discarded.

### High Frequency Monitoring of Phytoplankton Distribution with Special Reference to Cyanobacteria in a Freshwater Lake

Lohse, D.\* & Boehme, T.

*bbe Moldaenke GmbH, R&D Dept., Germany* \*DLohse@bbe-moldaenke.de

Lakes play a relevant role in the supply of raw water for drinking water processing as for recreation. Both utilizations need a high quality of water to guarantee the health of all involved parties. Algae blooms especially formed by cyanobacteria pose a threat to humans and animals. Here we investigated the development and remains of microalgae and cyanobacteria in Clear Lake, California, over two years of 2020/2022. Different techniques were used to monitor the water at a high frequency of measurements for phytoplankton composition, as for microcystin and unbound phycocyanin. A new fluorometric approach determined the release of the phycocyanin as a proxy for damaged cyanobacterial cells and the release of potential occurring biotoxins. As part of a pilot project, we provide on-site and satellite data to visualize the developments of phytoplankton distribution in the lake. This knowledge enables the control of water processing measurements and reflects the status of surface water in recreational areas along the lake.

# Adaptation to temperature extremes could improve marine forest restoration success. A case study for a unique, isolated *Gongolaria barbata* population in the northern Adriatic Sea

Smith, S.\*, Bilajac, A., Gljušćić, E. & Iveša, L.

Laboratory for the ecology of benthos, Ruđer Bošković Institute, Center for Marine Research Rovinj - Croatia \*ssmith@irb.hr

In the Mediterranean region, coastal development has caused major declines for intertidal seaweed forests, but ecosystem restoration has recently been identified as a promising way to recover and conserve lost marine forest habitat. Increases in sea urchin populations, however, currently limits restoration success and projected sea temperature extremes threaten the longevity of such projects. In the northern Adriatic Sea, the coldest part of the Mediterranean, we have identified a refuge population of the declining habitat forming species, *Gongolaria barbata* that, contrary to expectation, thrives in the extreme temperatures experienced in this unique environment. Here, we explored the potential to source donor individuals and/or "seed" material from this site to plan for successful long-term restoration projects and additionally, tested their susceptibility to grazing in two separate aquaria studies. *G. barbata* shows a lifecycle, typical of fucalean species, where deciduous branches form in the boreal Autumn and declining temperatures typically trigger the formation of reproductive material. We ran a thermal tolerance experiment which showed that when comparing control temperatures of 18°C with 15°C, 12°C and 5°C temperature treatments, there is no difference in the timing of fertility, but recruitment was most successful when individuals were maintained at 18°C. Photosynthetic activity was significantly reduced

at 5°C, however growth (i.e. changes in maximum height) and survival were not influenced by temperature. A complex result in the context of habitat restoration. Finally, herbivory experiments showed that *G. barbata* is susceptible to grazing by multiple taxonomic groups which remains the most immediate bottleneck to restoration success.

#### Non-native hosts of an invasive seaweed holobiont have more stable microbial communities compared to native hosts in response to thermal stress

Bonthond, G<sup>1,2</sup>., Neu, A.-K.<sup>1</sup>, Bayer, T.<sup>1</sup>, Krueger-Hadfield, S.<sup>3</sup>, Künzel, S.<sup>4</sup> & Weinberger, F.<sup>1\*</sup>

<sup>1</sup>GEOMAR Helmholtz-Centre for Ocean Research, Kiel, Germany <sup>2</sup>ICBM, Carl-von-Ossietzky University Oldenburg, Wilhelmshaven, Germany <sup>3</sup>Department of Biology, University of Alabama at Birmingham, Birmingham, Alabama, USA <sup>4</sup>Max Planck Institute for Evolutionary Biology, Plön, Germany \*fweinberger@geomar.de

Seaweeds are holobionts, meaning they are colonized by a microbial community which can be directly linked to their performance. This community is shaped by an interplay of stochastic and deterministic processes, including mechanisms which the host deploys to manipulate associated microbiota. The Anna Karenina Principle predicts that when a holobiont is exposed to suboptimal or stressful conditions, these host mechanisms may be compromised, leading to a relative increase of stochastic processes that may potentially result in the succession of a microbial community that is harmful to the host. Based on this principle, we used the variability in microbial communities (i.e., beta diversity) as a proxy for host control within the invasive holobiont *Gracilaria vermiculophylla* during a simulated invasion into a common garden. Our results demonstrate that at moderately elevated temperature (22 °C vs. 15 °C), both host performance and host control decline. Moreover, our data provide evidence that both host control and host performance are stronger in nonnative populations compared to native populations. Therefore, increased host control toward microbiota may have enhanced invasiveness of *G. vermiculophylla*, making this alga in the nonnative range more tolerant toward microbial pressures under a wider range of conditions.

### Harvesting *Turbinaria ornata* for coral reef restoration actions in French Polynesia

Tricoche, M.<sup>1,2</sup>, Tran, J.<sup>2</sup>, Bionaz, M.<sup>2</sup>, Harnay, P.<sup>2</sup>, Putnam, H.<sup>2,3</sup> & Zubia, M.<sup>1,2,4\*</sup>

<sup>1</sup>University of French Polynesia, BP 6570, 98702 Faa'a, Tahiti, French Polynesia
<sup>2</sup>TO'A NU'UROA consortium, <u>https://toanuuroa.org/</u>
<sup>3</sup>Department of Biological Sciences, University of Rhode Island, Kingston, RI 0288, USA
<sup>4</sup>Ekoalg, France
\*mayalenzubia@gmail.com

In French Polynesia, coral reefs are subject to a significant proliferation of the invasive brown alga, *Turbinaria ornata*. The bleaching event of 2019 has largely favored the invasion of *T. ornata* in Tahiti. In addition, pollution and overfishing exacerbate this effect and participate in the maintenance of *T. ornata*. It is present in all reef habitats and disperses by detaching from its substrate during strong swell events, forming floating masses that colonize Polynesian islands as far as the Tuamotu Archipelago. Its

proliferation directly threatens coral recruitment and thus the balance of coral reefs. The harvesting of these algae represents a solution to its invasion but also an opportunity to develop the industrial valorization of this resource in agriculture, cosmetic and biomaterial sectors. The association Tamari'i Pointe des pêcheurs has been working for 6 years on the restoration of degraded reefs by propagating coral fragments and regularly pulling out invasive algae. In partnership with Tamari'i No te Moana, the associations are joining forces with the University of French Polynesia, EkoAlg, Putnam Lab and Pacific Biotech to carry out the TO'A NU'UROA project supported by the French Biodiversity Agency. Over a period of 2 years (2023-2025), the objectives are: (i) To decrease the algal cover of *Turbinaria ornata* by manual removal and including it in a valorization process, (ii) To increase the coral cover by using microfragmentation techniques.

In the long term, the TO'A NU'UROA project aims to propose less expensive management measures and operational action protocols to restore coral reefs.

#### SYMPOSIUM 12 "Polar algae in a changing environment"

### Adaptation versus plastic responses to environmental controls in snow and glacier algal blooms

Broadwell, E.L.M.\*, Pickford, R.E. & Williamson, C.J.

School of Geographical Sciences, University of Bristol, United Kingdom \*emily.broadwell@bristol.ac.uk

Snowpack and supraglacial ice surface environments are home to a diverse assemblage of microorganisms, with predominately Chlorophyte snow algae and Streptophyte glacier algae acting as the prevalent photoautotrophs. Both groups form widespread algal blooms during the summer melt seasons, and these have been highlighted to have far-reaching consequences for both the physical and chemical characteristics of the supraglacial environment. Despite their potential importance however, our understanding of the diversity of adaptive capabilities within the snow and glacier algae to dominate their dynamic supraglacial environments remains diminutive.

Here we report on recent efforts to examine the photophysiology and stoichiometry across the snow algal phylogeny (*Microglena* sp. and *Chloronomas* sp.) relative to light, temperature, and nitrate availability. Results demonstrate how cold temperatures can mimic the impacts of high light on photophysiology, as well as a preference for the *Chloromonas* sp. strains for higher light intensities. The stoichiometry (C:N) of the snow algal strains also approached Redfield when grown under nutrient replete conditions, significantly lower to that achieved when grown under *in-situ* nitrate concentrations. Our lab incubations were a strong foundation to try and constrain the amount of dynamism of snow algal species relative to light, temperature, and nutrient concentrations *in-situ*. Over the last two field seasons we have investigated these parameters of both snow and glacier algal blooms in alpine and high-arctic environments. Here we will present how these findings compare both with each other, and to the trends originally uncovered during the original laboratory incubations.

#### A draft genome of the snow algae Sphaerocystis sp.

Ciftci, O.<sup>1\*</sup>, Zervas, A.<sup>2</sup>, Lutz, S.<sup>1,3</sup>, Leya, T.<sup>4</sup>, Keusching, C.<sup>1</sup>, Tranter, M.<sup>2</sup>, Anesio, A.M.<sup>2</sup> & Benning, L.G.<sup>1,5</sup>

<sup>1</sup>Interface Geochemistry, GFZ German Research Centre for Geosciences, Potsdam, Germany <sup>2</sup>Department of Environmental Science, Aarhus University, Denmark <sup>3</sup>Plant-Soil Interactions, Department of Agroecology and Environment, Agroscope, Zurich, Switzerland <sup>4</sup>Extremophile Research & Biobank CCCryo, Fraunhofer Institute for Cell Therapy and Immunology, Branch Bioanalytics and Bioprocesses (IZI-BB), Potsdam, Germany. <sup>5</sup>Department of Earth Sciences, Free University of Berlin, Germany \*ozan.ciftci@gfz-potsdam.de

Pigmented snow algae are important primary producers in polar and alpine ecosystems. Various snow algal phenotypes thrive on semi-permanent and permanent snow fields where they experience harsh conditions, such as low temperatures, high irradiation, and often oligotrophic nutrient levels. They form colourful blooms that can reduce the snow surface albedo and enhance melting. Although their physiology, biogeographical distribution and diversity are well described, snow algae genome data is lacking. This hampers our ability to understand their evolutionary dynamics, metabolic potential and cellular responses to changing environmental conditions and to predict their roles in future global warming climate scenarios.

To fill this knowledge gap, we have assembled the nuclear genome of the psychrophilic snow alga *Sphaerocystis sp.* We used a hybrid long-read assembly of Pacbio (~14 Gb) and Nanopore (~ 2 Gb) data and polished these with short-read Illumina (~8 Gb) data to produce a ~120 Mb assembly (%GC = 53) with 93% BUSCO completeness. Our draft genome consists of more than 600 contigs with an N50 around 900 kbp. About 1/4 of the total length of our genome assembly was composed of repeats, a proportion similar to the mesophilic chlorophyceaen algae genomes. Our draft assembled snow algae genome and ongoing work on gene annotations will allow in depth comparisons with the increasing number of available algae genomes and thus improve our understanding of cryogenic eukaryotic microbiology and algal adaptation to snow habitats.

#### Intertidal macroalgal communities in the European Arctic

Düsedau, L.<sup>1,2\*</sup>, Savoie, A.<sup>3</sup>, Fredriksen, S.<sup>4</sup> & Bartsch, I.<sup>1</sup>

<sup>1</sup>Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research, Bremerhaven, Germany
 <sup>2</sup>BreMarE, University of Bremen, Bremen, Germany
 <sup>3</sup>Centre for Arctic Change and Exploration, Canadian Museum of Nature, Ottawa, Canada
 <sup>4</sup>Department of Biosciences, University of Oslo, Oslo, Norway
 \*luisa.duesedau@awi.de

Kongsfjorden in western Svalbard is a high Arctic fjord with numerous glaciers in transition. As ocean warming continues, the fjord is undergoing rapid changes due to glacier and sea ice retreat. In contrast to that, Porsangerfjorden in Finnmark is a more southern Arctic fjord without glaciers and only restricted ice coverage in winter. Macroalgal communities in both fjords form the basis of the local coastal marine ecosystems. In a space-for-time-approach, we compare macroalgal biodiversity and biomass in the shallow subtidal and intertidal to understand how macroalgal communities in the high Arctic are likely to develop in future. Quantitative sampling was performed at the infralittoral fringe level (n=3, 0.5x0.5m frames) in July 2021 (Kongsfjorden) and July 2022 (Porsangerfjorden). In this zone macroalgal species composition is more diverse in Porsangerfjorden than in Kongfjorden (23 vs 16 morpho-species), but biomass dominant species and mean fresh biomass are similar at both sites. Stratified random sampling in the low, mid and high littoral zone (n=3, 0.5 x 0.5 m frames) along the fjord axis of Porsangerfjorden in summer 2022 revealed a strong alongfjord gradient in intertidal macroalgal biomass with a maximum fresh biomass of 20 kg m<sup>-2</sup>. Preliminary data show that intertidal macroalgal communities on Svalbard

are largely different. Porsangerfjorden is dominated by seven cold-temperate to Artic Fucales species while in Kongsfjorden this group is only represented by one species. We aim to highlight the shifts in macroalgal richness and changes in productivity to predict possible ecosystem changes along Arctic shores.

### Warming modifies the seasonal photophysiology and productivity of Arctic macroalgae

Iñiguez, C.<sup>1</sup>\*, Carmona, R.<sup>1</sup>, Gordillo, F.J.L.<sup>1</sup>, Gordo, E.<sup>2</sup>, Cañete, S.<sup>2</sup> & Smerdou, C.<sup>1</sup>

<sup>1</sup>Department of Ecology, Faculty of Sciences, University of Málaga (Spain) <sup>2</sup>SCAI, Central Research Facilities, University of Málaga (Spain) \*iniguez@uma.es

Warming is affecting Kongsfjorden ecosystem with special intensity due to the influence of oceanic currents altered by Global Change. The effects of this stressor on the ecophysiology of Arctic seaweeds have been widely investigated, but mostly restricted to summer. However, Arctic coastal ecosystems experience strong seasonal changes in environmental light conditions from 24-hours of darkness in winter to 24-hours of light in summer, which likely alter the photosynthetic performance of macroalgae. In order to understand how increasing temperature will affect Kongsfjorden ecosystem dynamics it is crucial to analyze the effect of seasonal photoperiod on the responses of Arctic seaweeds to warming. Thus, we carried out experiments in September (fall equinox), March (spring equinox) and August (24h of light) to compare the photophysiological responses of common seaweed species of Kongsfjorden after acclimation to continuous light and 12:12 light:darkness at 4°C, as well as the responses to increased temperature (8°C) at the corresponding seasonal photoperiod.

Due to 24-hours light stress in summer, macroalgae generally showed reduced photosynthetic capacity when compared to the equinoxes. Additionally, higher photoinhibition along with higher respiration rates were induced when seaweeds were exposed to continuous light in the equinoxes, whereas macroalgae exposed to 12:12 light/dark cycles in summer showed no changes in the photosynthetic capacities and respiratory rates, indicating that seaweeds cyclically acclimate to the seasonal light conditions in the Arctic. These differences were observed when photosynthetic light reactions were assessed, but were reduced or even disappeared when <sup>14</sup>CO<sub>2</sub> fixation was measured. The increase in temperature frequently enhanced <sup>14</sup>CO<sub>2</sub> fixation and respiration rates, while growth rates were mostly unaltered, but season-specific and species-specific effects were observed. These results are highly valuable for constructing primary productivity models of the macrophytobenthos for the whole fjord, which can serve to make accurate predictions of productivity and ecosystem functioning in near-future scenarios.

### Low-temperature outdoor cultivation of polar microalgae: a comparison of tests in central Europe and Svalbard

Kvíderová, J.<sup>1,2,3</sup>, Elster, J.<sup>1,2</sup>, Šnokhous, K.<sup>4</sup>, Bělohlav, V.<sup>4</sup>, Jirout, T.<sup>4</sup>, Lukavský, J.<sup>1</sup>, Přibyl, P.<sup>1</sup>, Liška, J.<sup>1</sup> & Nedbalová, L.<sup>1,5</sup>\*

<sup>1</sup>Centre for Phycology, Institute of Botany, The Czech Academy of Sciences, Třeboň, Czech Republic <sup>2</sup>Centre for Polar Ecology, Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic <sup>3</sup>Centre for Biology, Geosciences and the Environment, Faculty of Education, University of West Bohemia, Plzeň, Czech Republic <sup>4</sup>Department of Process Engineering, Faculty of Mechanical Engineering, Czech Technical University in Prague, Prague, Czech Republic <sup>5</sup>Department of Ecology, Faculty of Science, Charles University, Prague, Czech Republic \*lindane@natur.cuni.cz

The complex adaptation of polar microalgae makes them prospective candidates for sustainable lowtemperature biotechnology. To test the outdoor production of biomass and high-value compounds, we focused on a set of strains of green algae (Chlorophyta) from our working collection (namely from the genera *Monoraphidium, Bracteacoccus, Chodatodesmus* and *Neocystis*) that were isolated from freshwater, snow and soil samples collected in Svalbard (High Arctic) and James Ross Island (Antarctica).

An open thin-layer photobioreactor (volume 150 L) was successfully used for biomass production in winter conditions of central Europe. Furthermore, a closed flat panel type bioreactor (volume 20 L) equipped with an automated rotation mechanism that follows the Sun was recently developed for the cultivation in the High Arctic. It has been designed to resist to extreme operating conditions and allows to optimalise the capture of photosynthetically active radiation under conditions, when light represents the main factor limiting algal growth (winter period in temperate zone, summer in polar regions). An Arctic strain of the coccoid alga *Neocystis mucosa* (Trebouxiophyceae) isolated from soil was selected for the pilot tests of the bioreactor that were performed in the Czech Republic and on Svalbard, enabling comparison of growth rate and photosynthetic performance of algae under different conditions and also with the thin-layer photobioreactor. These tests must be followed by a multi-criteria evaluation considering technical, environmental, and economic aspects at industrial scale.

### Terrestrial run-off reduces the quality of Arctic kelps as a food source

Niedzwiedz, S.<sup>1\*</sup>, Schmidt, C.E.<sup>2</sup>, Hildebrandt, L.<sup>3</sup>, Pröfrock, D.<sup>3</sup>, Thomas, H.<sup>2</sup>, Zhang, R.<sup>4</sup>, Yang, Y.<sup>4</sup>, Andersen, S.<sup>5</sup>, Damsgård, B.<sup>5</sup> & Bischof, K.<sup>1</sup>

<sup>1</sup>Marine Botany, Faculty of Biology and Chemistry & MARUM, University of Bremen, 28359 Bremen, Bremen, Germany

<sup>2</sup>Institute of Carbon Cycles, Helmholtz-Zentrum Hereon, Max-Planck-Straße 1, 21502, Geesthacht, Germany <sup>3</sup>Department for Inorganic Environmental Chemistry, Helmholtz-Zentrum Hereon, 21502, Geesthacht, Germany

<sup>4</sup>State Key Laboratory of Marine Environmental Science, Xiamen University, Xiamen 361005, China <sup>5</sup>The University Centre of Svalbard (UNIS), Longyearbyen NO-9171. Norway

\*sarina@uni-bremen.de

Kelps are ecosystem engineers along polar rocky coastlines. Currently, Arctic kelps are experiencing various dramatic environmental changes. As a consequence of temperature increase, glacial melt results in high concentrations of terrestrial sediments being washed into the fjords, altering its physical and chemical parameters.

We conducted an interdisciplinary approach to assess how terrestrial run-off affects the quality of kelps as a food source to associated fauna, by determining their elemental composition and associated microbial community. We chose Billefjorden, Svalbard as a model system, as it hosts areas being characterized by 1) glacial melt, 2) terrestrial run-off and 3) relatively clear coastal water (control) in close proximity.

In each area, we mapped the physical parameters and took water samples to measure the concentration of dissolved elements ( $<45 \mu m$ ). We found a clear signal of meltwater presence in areas 1) and 2),

becoming evident by reduced salinities, higher light attenuation coefficients and changes in the composition of dissolved elements. Along with the water samples, we collected kelp specimens (*Saccharina latissima*) and detected a significant higher concentration of various elements (e.g. Al, Hg, Pb, Cu) in kelps growing in meltwater presence. The accumulation of heavy metals reduces the quality of kelps as a food source, with potentially drastic consequences for higher trophic levels. Furthermore, area specific differences in kelp associated microbial communities have been assessed to draw conclusions about the provision of secondary metabolites for grazers. These data might have implications toward the potential implementation of kelp mari-cultures in high Nordic fjord ecosystems.

### Metabolic characterization of glacier ice algae dominated microbial communities blooming on the Greenland Ice Sheet

Peter, E.K.<sup>1,2</sup>\*, Jaeger, C.<sup>3</sup>, Lisec, J.<sup>3</sup>, Peters, R.S.<sup>1</sup>, Mourot, R.<sup>1,2</sup>, Rossel, P.E.<sup>1</sup>, Tranter, M.<sup>4</sup>, Anesio, A.M.<sup>4</sup> & Benning, L.G.<sup>1,2</sup>

<sup>1</sup>Interface Geochemistry, German Research Centre for Geosciences, Germany <sup>2</sup>Department of Earth Sciences, Free University of Berlin, Germany <sup>3</sup>Federal Institute for Materials Research and Testing, Germany <sup>4</sup>Department of Environmental Science, Aarhus University, Denmark \*elisa.peter@gfz-potsdam.de

The bare ice surface of the Greenland Ice Sheet is inhabited by active microbial communities, dominated by the glacier ice algae *Ancylonema alaskanum* and *Ancylonema nordenskiöldi*. These algae significantly decrease surface albedo due to their purple-brown pigmentation, thus increasing melt. To date, we lack knowledge of glacier ice algae growth dynamics and how they may reinforce melt progression in a warming climate. Metabolomics is a promising approach to directly capture the physiochemical state of glacier ice algae and determine biological and physical parameters controlling their growth and pigment production. However, there is currently no data characterizing glacier ice algae metabolomes, largely because we lack validated sample processing and analytical workflows tailored to this unique extreme environment, where sample melting could significantly influence metabolite composition.

We used an untargeted, high-resolution mass spectrometry-based approach and tested the effect of temperature and duration of sample melting prior to quenching on the metabolome and lipidome of algae rich surface ice samples. We putatively identified 817 metabolites, with glycerolipids, glycerophospholipids and fatty acyls being the most prominent superclasses (>50 % of identified metabolites). Carbohydrates and amino acid derived metabolites were the most abundant among the polar metabolome. We show that 7.8 % of the metabolome was affected by melt duration at low temperature, with a pronounced effect on betaine membrane lipids and pigment precursors, which decreased with melt duration, while phospholipids increased. Fast sample melting in a temperature-controlled water bath resulted in highest consistency and is our recommendation for future glacier ice algae metabolomics studies.

### Biological soil crust microalgae manipulation experiments in the High Arctic

Hejduková, E.<sup>1,2</sup>, Paterová, A.<sup>3</sup>, Frank, E.<sup>3</sup>, Přibyl, P.<sup>2</sup>\*, Nedbalová, L.<sup>1,2</sup> & Elster, J.<sup>2,3</sup>

<sup>1</sup>Department of Ecology, Faculty of Science, Charles University, Viničná 7, CZ–12844 Prague 2, Czech Republic,

<sup>2</sup>The Czech Academy of Sciences, Institute of Botany, Dukelská 135, CZ–37982 Třeboň, Czech Republic,

<sup>3</sup>Centre for Polar Ecology, Faculty of Science, University of South Bohemia, Na Zlaté stoce 3, CZ–37005 České Budějovice, Czech Republic

\* pavel.pribyl@ibot.cas.cz

Biological soil crusts (BSC) are communities of organisms found in the upper layer of soil in arid and semi-arid areas from all over the world. In comparison to their counterparts from the Artic tundra, crusts of High Arctic polar deserts are often dominated by microalgae. Arctic soil crusts have received far less attention, and represent an environment characterised by extreme natural conditions microalgae must overcome.

The field experiment in the High Arctic was established in the summer 2022 and focuses on BSC microalgae resistance to stresses associated with seasonal Arctic conditions and climate change. "Hardening" of algae and cyanobacteria by nutrient starvation, cold and/or desiccation was reported to increase stress tolerance. Such microalgae appeared to be more resistant to freeze-thaw cycles, freezing and desiccation stress in comparison to those growing under more favourable conditions. The environment in the field was manipulated by watering and fertilization (summer) and additional watering and artificial thawing to simulate rain-onsnow events and winter warm spells (the upcoming winter).

The photosynthetic and physiological activity of the microalgae was tested by measurements of photosynthetic parameters. Field observations and manipulation studies will be linked to additional laboratory experiments and analyses focused on physiological, morphological and ultrastructural observations. Likewise, metagenomic and (meta)transcriptomic profiles will be analysed since most adaptation/acclimation mechanisms are connected to metabolic changes. The combination of molecular biology and microalgal physiology methods together with field studies will allow the complex insight into survival strategies of BSC microalgae, from sub-cellular to community levels.

### The physiological response of the Arctic haptophyte *Phaeocystis pouchetii* to marine heatwaves

Urbschat, N.<sup>1</sup>, Wolf, K.E.K.<sup>2</sup>, Rokitta, S.<sup>1</sup>, Hoppe, C.J.M.<sup>1</sup> & Rost, B.<sup>1\*</sup>

<sup>1</sup>Marine Biogeosciences, Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research, Bremerhaven, Germany

<sup>2</sup>Institute of Marine Ecosystem and Fishery Science, Universität Hamburg, Hamburg, Germany

\* bjoern.rost@awi.de

Marine heatwaves (MHWs) are projected to increase in number, intensity and duration with further global warming, especially for the Arctic Ocean. Such temperature extremes can push a species beyond their usually experienced temperature range, and they arise and disappear faster than the timeframes needed for acclimation or adaptation. We here hypothesized that temperature fluctuations associated with MHWs cause temporal mismatches of photosynthetic processes, which in turn lead to the accumulation of reactive oxygen species (ROS) and lowered fitness of cells.

In a laboratory experiment, we exposed the Arctic phytoplankton species *Phaeocystis pouchetii* to MHWs with a temperature increase of 3 °C (3 °C  $\rightarrow$  6 °C) and a duration of 6 vs. 10 days, followed by a 5-day recovery phase at 3 °C. Responses of cells were assessed by measuring specific growth rates, elemental composition and cellular chlorophyll *a* content, but also photo-physiological assays as well as measurements of intracellular levels of O<sub>2</sub><sup>-</sup> and H<sub>2</sub>O<sub>2</sub> using flow cytometric analyses.

Warming strongly stimulated growth rates in the short-term, but had no significant effect on biomass production rates. As heat exposure continued, photosynthetic efficiency was reduced and ROS accumulated in the cell. In the long-term, *P. pouchetii* was not able to maintain the stimulated growth rates, which could be explained by a reallocation of energy into ROS detoxification. Interestingly, cooling to 3 °C after the MHWs represented a relief to cells in regard to oxidative stress, although it decreased biomass production rates significantly.

### Diversity and distribution of Arctic protists in pelagic and sympagic habitats in the northern Barents Sea as revealed by metabarcoding

Saubrekka, K.<sup>1\*</sup>, Vader, A.<sup>2</sup>, Supraha, L.<sup>1</sup>, Andersen, T.<sup>1</sup> & Edvardsen, B.<sup>1</sup>

<sup>1</sup>University of Oslo, Norway <sup>2</sup>University of Svalbard, Norway \*karoline.saubrekka@ibv.uio.no

Protists have various crucial roles in the marine ecosystems and in a rapid changing Arctic it is important to address who's there, when and where, for future management and research of the northern Barents Sea. The main objective in this study is to describe and compare the composition of the protist community in sea ice and pelagic habitats of samples collected along a S-N transect in the northern Barents Sea during in August 2018 with low sea-ice extent and 2019 with sea-ice extent high. By using metabarcoding targeting the 18S rRNA gene we revealed 3682 unique Amplicon sequence variants (ASV), a proxy for a species. The data shows a clear separation of ASV composition in the ice- and pelagic habitats. The pelagic community showed interannual differences along the sampling transect. In the year 2018 the community was dominated by dinoflagellates and nano- and pico-plankton such as Micromonas polaris. In the year 2019 a community with more centric diatoms was observed at station in the ice edge. Members of the class Chrysophyceae dominated in the melt ponds, whereas the community composition within the ice (dominated by dinoflagellates and pennate diatoms) changed from homogeneous to heterogeneous with increased ice cores thickness. Right below the ice other dinoflagellates ASVs were dominating, together with Phaeocystis. These results indicates that protist communities are distinct and specialized on habitat. With further ocean warming the pelagic community could shift towards smaller flagellates and thinning of the sea ice may result in loss of habitat niches diversity.

#### **SYMPOSIUM 13 "Taxonomy and Systematics"**

### De novo transcriptome characterization of Ulva lacinulata under in situ emersion/immersion cyclic conditions

Parages, M.L.<sup>1\*</sup>, Sánchez de Pedro, R.<sup>2</sup> & Smerdou, C.<sup>1</sup> (formerly Jimenez, C)

<sup>1</sup>Department of Ecology, Faculty of Sciences, Málaga, Spain <sup>2</sup>Department of Botany and Plant Physiology, Faculty of Sciences, Málaga, Spain \*malopa@uma.es

The green algal genus *Ulva* Linnaeus (Ulvaceae, Ulvales, Chlorophyta) displays a worldwide distribution in marine, freshwater and brackish ecosystems, and are really well adapted to fluctuating natural environments. Despite increasing interest on the analysis of the ecophysiological responses

showed by organisms to face environmental shifts, knowledge of the genetic and molecular mechanisms underlying those responses are still scarce. These responses determine the survival of organisms under pressure of different environmental stresses and the regular ecosystem behaviour. In order to disentangle the genetic networks that might regulate the adaptation mechanisms of these organisms in a changing environment, the characterization of the *de novo* transcriptome from *Ulva lacinulata* derived from a coastal ecosystems of southern Spain under *in situ* cyclic conditions of emersion/immersion by using Next Generation Sequencing technologies was carried out. Transcriptome sequencing and transcript-level expression analysis were performed by Illumina®NextSeq® 550 system platform. A total of 100,251 unigenes were expressed during emersion/immersion process. Based on the differentially expressed genes (DEGs), genes associated with different biosynthetic metabolic pathways were annotated according to Gene Ontology and Kyoto Encyclopedia of Genes and Genomes Orthology (KEGG). These findings shed light on the molecular mechanisms underlying rapid and successful ecophysiological response of marine macroalgae in cyclic tidal conditions.

## SYMPOSIUM 14 "Algal culture collections: their increasing societal relevance and role as gene repositories"

#### Algal bioprospecting using Machine Learning on Omics data

Iha, C.\*, Lee, E. & Willis, A.

Australian National Algae Culture Collection, CSIRO, Australia \*cintia.iha@csiro.au

Interest in algal bioproducts is increasing worldwide because algae have been recognised as a novel, sustainable and ethical sources of bioproducts. Many algal species have been explored for aquaculture, animal feedstock, human consumption, pharmaceutical, energy, and other industrial activities. Algae biodiversity is incredibly high, and a wealth of bioproducts are of growing interest to various industries. A vast amount of new bioproducts is anticipated to be discovered, with countless species yet to be explored.

The systematic search for sources of bioproducts is the most challenging and timeconsuming task in bioprospecting. However, applying machine learning (ML) algorithms linking detailed omics characterisation, such as genomics, proteomics, and metabolomics, with the evolutionary relationships of algae can be a critical ally to predicting potential new bioproducts and allowing predictive identification of algae for targeted bioproducts. This study proposes building a comprehensive multiomics database with 500 algae species comprising wide taxonomical diversity and developing an algae bioprospecting platform to rapidly identify algae sources of bioproducts.

The Great Southern Reef (GSR) is a seaweed biodiversity hotspot in which at least half of the algae species are uncharacterised, and their bioproducts are unknown. Our database will focus on (but not be limited to) GSR species and ones cultivated at the Australian National Algae Culture Collection. The immediate output from information gathered with this multiomics database will be a treasure trove for future algal research. This combination of multiomics data with ML is unique and represents a resource to accelerate future product development and biodiscovery.

#### **CCAC - Central Collection of Algal Cultures**

#### Marks, S.

CCAC, Biology, University Duisburg-Essen, Germany Sabina.marks@uni-due.de

The "Central Collection of Algal Cultures (CCAC)" at the University of Duisburg-Essen (UDE) is one of the largest collections of algal cultures worldwide. The responsibility for the maintenance and development of the CCAC was taken over by the UDE from the University of Cologne (formerly Culture Collection of Algae at the University of Cologne), the relocation of the collection to Essen was completed in January 2020. The CCAC maintains approximately 7500 strains from around the world in five modern walk-in growth chambers operated at different temperatures and LED-illumination (2 x 15°C, 1 x 20°C, 1 x 23°C, 1 x 4°C). The strains are clonal or single cell isolates. Most of the strains in the CCAC derive from freshwater/terrestrial habitats (85%), the remaining 15% represent marine or brackish water strains. 15% of the strains are axenic. More than 6000 strains are currently available to the public.

### The use of *Ulva* as a final stage nutrient removal from factory effluent water

Nelly, A.\*, Inaba, M., Simon, C., Goulding, D., Ryan, I., McCarthy, J., McHale, M. & Sulpice, R.

Plant Systems Biology Lab, Ryan Institute & MaREI Centre for Marine, Climate and Energy, School of Biological & Chemical Sciences, University of Galway, H91 TK33 Galway, Ireland A.Nelly1@nuigalway.ie

*Ulva*'s rapid growth rates and tolerance to varying environments has gained much attraction in recent years, resulting in growing use of *Ulva* in aquaculture and bioremediation applications. Such properties of *Ulva* spp. can be advantageous especially in the presence of nutrient rich waters. *Ulva spp.* offers the possibility to remove nutrients from wastewaters, mitigating eutrophication, while providing valuable biomass for diverse industrial applications. Current wastewater treatment systems involve various long and complex approaches in order to remove both soluble and insoluble contaminants from industrial waste and *Ulva* spp. might be a cheap and efficient alternative, or at least be used to complement part of the current processes.

We sought to identify strains/species with improved low salinity tolerance to support applications with typical terrestrial wastewater streams. To this end, we co-cultivated multiple strains from each of three species of foliose *Ulva* under three different conditions: low-salinity (17.5 ppt artificial seawater with F/2 nutrients), wastewater dilution (artificial seawater diluted with industrial wastewater to ~17.5ppt) and nutrient supplemented wastewater dilution (wastewater dilution with additional F/2 nutrients). Samples of biomass are being collected regularly over a period of 8 weeks and CAPS assays are being performed to determine species composition over time. Further, biochemical assays are being performed to characterise impacts of selection under each condition on the quality of biomass produced.

### The Culture Collection of Algae and Protozoa (CCAP): preserving diversity for algal research and biotechnology

Rad-Menéndez, C.\*, Allen, R., Drysdale, C., Field, J., MacKechnie, K., Rowell, J., Saxon, R., Thomas, N. & Ross, M.

*Culture Collection of Algae and Protozoa (CCAP), Scottish Association for Marine Science, Oban, PA371QA, Scotland, UK* 

\* cecilia.radmenendez@sams.ac.uk

The Culture Collection of Algae and Protozoa (CCAP) is a biological resource centre comprising a wide range of biodiversity, with strain holdings spanning Prokaryotic cyanobacteria to most Eukaryotic lineages. The origins of our strains correspond to a broad temporal and spatial diversity of environments. The collection comprises over 3000 strains in the public domain of which 350 are Type strains. CCAP specialises in the isolation, maintenance, identification and distribution of cultures and their associated data, as well as the molecular identification and taxonomic revision of strains.

CCAP has a long history of research relating to cryopreservation. Cryopreservation offers a great solution to space and manpower limitations in culture collections through establishment of cryopreserved biobanks that enable the *ex-situ* preservation of a bigger genetic diversity for future research. To date, many of the strains in the collection have been cryopreserved, including microalgae, macroalgae and protozoa. CCAP collaborates with a broad range of algal research projects including several protist's genome projects, with protists making up most of the genetic complexity of our planet's ecosystems, CCAP aims to contribute and support research into this diverse and ecologically important group of organisms.

Culture collections, such as CCAP, have huge ecological and economic importance. They are key for the preservation of genetic diversity to support and contribute to protistan research, biotechnology, and natural resource management through *ex-situ* conservation of diversity that might be able to regenerate species that are lost to their natural habitat.

### Algae biobanking : cryopreservation at the Australian National Algae Culture Collection

Watson, R.J.\*, Lee, E., Willis, A. & Jameson, I

Australian National Algae Culture Collection, CSIRO, Australia \*ros.watson@csiro.au

At the Australian National Algae Culture Collection (ANACC), we maintain ~1100 living strains, most of Australian origin, that we provide to industry, Universities and research. Living strains are maintained through serial sub-culturing, while this provides ready access to cultures it requires extensive maintenance and laboratory support. Expanding a culture collection involves both additional staff and growth spaces. Cryopreservation has been used by major algae collections for backing up their living collections, managing ongoing maintenance costs and minimizing genetic drift in cultures. Our newly established biobank features strains from several algal classes cryopreserved using Mr Frosty with DMSO as the cryoprotectant.

The success of recovery from frozen cultures varies depending on the strain. To optimize recovery, we monitored the growth of 54 cryopreserved strains using a Biotek Cytation plate reader. We assessed the effects of varied light introduction and thawing protocols on postthaw culture growth. We found culture recovery at the taxa level was more varied than at strain level and that few strains exhibited a significant

change in recovery when comparing a short light acclimation of 24hrs to a longer stepwise light acclimation over 3 days.

## SYMPOSIUM 15 "Algae as producers of valuable compounds"

#### Protein potential of a Portuguese Spirulina strain

Aksoy, B.\*, Assunção, M.F.G & Santos, L.M.A.

Coimbra Collection of Algae (ACOI), Department of Life Sciences, University of Coimbra, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal \*berk.aksy00@gmail.com

According to the United Nations, the Earth population in 2050 will be 9.7 billion, therefore new food sources need to be searched for. The potential of microalgae for human and animal consumption and health is enormous due to their macromolecule composition. Also, microalgae are highly rich in bioproducts with similar or higher values than plants. *Spirulina* has been used for human consumption for centuries, with records dating back to the Middle Ages. It has received increased attention by researchers mainly for its protein content (50-71% DW). So far, the *Spirulina* strains used for commercial purposes and present in the Portuguese market come from other countries. In this work, we characterize the protein content of a *Spirulina* strain collected and isolated from a Portuguese marsh as well as optimize the cultivation conditions in order to obtain maximum productivity.

*Spirulina* sp. ACOI 1376 was cultivated in a 16 L photobioreactor with controlled conditions for 7 days. To obtain maximum productivities light intensity, photoperiod, medium pH and temperature were manipulated. Protein content was determined and compared by four methods, Bradford and Lowry assays, Kjeldahl method and elemental analysis.

Biomass productivities decreased with the increase of temperature above 30 °C as well as with the increase of light intensity above 79  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>. The protein content ranges from 40 to 65% DW depending on the cultivation conditions.

In conclusion, this *Spirulina* strain seems to be a good candidate for the market, especially for Portuguese producers who are concerned about the importance of using native species.

#### Unlocking a nature treasure-chest: the lipid profile of Eustigmatophyceae

Assunção, M.F.G\*, Lima, T., Aksoy, B. & Santos, L.M.A.

Coimbra Collection of Algae (ACOI), Department of Life Sciences, University of Coimbra, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal \*mariana.assuncao@uc.pt

Eustigmatophyceae is one of the classes of the large algal phylum Ochrophyta, still dealing with challenges related to diversity and taxonomy. This class has been the focus of bioeconomical interest due to the tendency of well-known genus *Nannochloropsis* to accumulate large amounts of lipids, including polyunsaturated fatty acids. Recent studies revealed more diversity and phylogenetic relationships within the class, however, the lipid profile of new representatives has not yet been

explored. With this study, we intend to make a major step towards unveiling the lipid and fatty acid profile of representatives of each known clade of the Eustigmatophyceae class.

Fourteen strains were selected and cultivated under controlled conditions during 10 and 30 days. Total lipid was extracted and fractionated into neutral lipids, glycolipids, and phospholipids. The fatty acid profile of each lipid fraction was determined by gas chromatography.

In general, the total lipid content decreased with the increase of cultivation time. The highest lipid class obtained was that of neutral lipids, followed mainly by glycolipids and phospholipids at both cultivation times. The fatty acid profile of neutral lipids is composed mainly by saturated fatty acids (C14:0 and C16:0), the glycolipids by polyunsaturated fatty acids (C20:5 $\omega$ 3) and the phospholipids by saturated (C16:0) and monounsaturated fatty acids (C16:1).

In conclusion, this work paints a rich picture of the lipid content of one of the most promising classes of algae demonstrating the potential of other lineages besides the one comprising the genus *Nannochloropsis*.

### High hydrostatic pressure assisted antimicrobial extraction from fucoid *Ericaria selaginoides*

Aymerich, T.<sup>1\*</sup>, Ray, S.<sup>1</sup> & Peteiro, C.<sup>2</sup>

<sup>1</sup>Institute of Agrifood Research and Technology (IRTA), Food Safety and Functionality Programme, Finca Camps I Armet s/n 17121 Monells, Girona (Spain).

<sup>2</sup>Spanish Institute of Oceanography of the Spanish National Research Council (IEO, CSIC), Oceanographic Centre of Santander (COST), Marine Culture Units "El Bocal", Seaweeds Centre. Barrio Corbanera s/n, 39012 Monte, Santander (Spain).

\*teresa.aymerich@irta.cat

Nowadays there is an increasing interest for natural versus synthetic additives to produce safe food supplies with high quality and longer shelf-life. A driving force for food industry innovation to develop alternatives solutions for food safety. In these sense brown algae, with compositional characteristics described to contain potential antimicrobial compounds, are promising alternatives sources.

Nevertheless, to food application, there is a need to obtention optimized foodgrade extracts that ensures preservation of the safe active valuable extracts. In this way, alternative technologies such as high hydrostatic pressure (100-1000 MPa), a non-thermal technology used in food industry, has emerged as a versatile technology, also to be used for extraction of active compounds from plants and red algae in a quicker and more sustainability way.

The aim of this study was to assess the effect of high hydrostatic Technology (HHP) to improve extraction of antimicrobials compounds from the brown algae, *Ericaria selaginoides* against *Listeria monocytogenes*.

Macroalgae mid-polarity extracts were obtained from *E. selaginoides*, a fucoid species collected from the Northwest of Spain and *Listeria monocytogenes*, still one of the most concerning food-borne pathogens due to associated highest mortalities rates, was used as a target strain for antimicrobial activity.

The results of our study showed that although HHP did not significantly change the mass dry yield of the crude extracts, in general, it significantly improved the antimicrobial activity of the extracts against *L. monocytogenes* as showed by the 2 to 4-fold decrease of the MIC (minimal inhibitory concentration).

#### Screening of cyanobacteria for their ability to produce bioproducts

Berrendero Gómez, E.<sup>1\*</sup>, García Abad, L.<sup>1</sup>, Martínez Gutiérrez, C.<sup>1</sup> & García, J.<sup>2</sup>

<sup>1</sup>Divertecnoalgae Research Group, Department of Applied Biology, Agro-food and Agroenvironmental Research and Innovation Center (CIAGRO-UMH), Miguel Hernández University, Elche, E-03312 Alicante, Spain.

<sup>2</sup>*GEMMA*-Group of Environmental Engineering and Microbiology, Department of Civil and Environmental Engineering, Universitat Politècnica de Catalunya. BarcelonaTech (UPC), c/ Jordi Girona 1-3, Building D1, E-08034 Barcelona, Spain.

\*eberrendero@umh.es

Cyanobacteria use light as an energy source and carbon dioxide as a primary source of carbon. In addition, they have a great capacity to adapt to the environmental changes that they have allowed them to live under extreme environmental conditions of radiation, drought and salinity. This adaptation has been possible thanks to the acquired unique metabolic properties by natural selection and synthesis of secondary metabolites. In recent years, there being increased the interest in the potential industrial applications of cyanobacterial metabolites. This is due to the growing demand for new high-value products that are synthesized in environmentally sustainable processes. However, the potential of cyanobacteria to contribute to the blue bioeconomy based the production of medium/low-value products is still unknown.

In the framework of the project CYANO2BIO, we are searching for new cyanobacterial strains with great capacity for producing biopolymers as PHBs. In this way, several cyanobacterial populations have collected from different environments ranging from rivers to soils, including brackish waters. Around forty cyanobacteria were isolated and each strain is being characterised by polyphasic approach, including morphological and molecular methods.

In collaboration with partners of the consortium, the strains will be assessed for their capacity for producing biopolymers as PHB. Subsequently, cyanobacteria with higher productivity for the target compounds will be selected for being cultured in synthetic medium strict-controlled closed photobioreactors for studying their optimization in terms of growth and biopolymers production.

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#### Diatom frustules – tools for vitamin encapsulation and release

Blaszczyk, A.<sup>1\*</sup>, Nilsson, S.<sup>2</sup>, Al-Handal, A.Y.<sup>1</sup> & Wulff, A.<sup>1</sup>

<sup>1</sup>Biological and Environmental Sciences, University of Gothenburg, Sweden <sup>2</sup>Swedish Algae Factory, Sweden \*gusblasad@student.gu.se

The need for marine-derived products - harvested sustainably, combining biology, biotechnology, and environmental sciences - is an area of growing interest. Industrial applications include for example pharmaceutical, cosmetic or nutraceutical products. Diatoms with their unique nanoporous "shell", the frustule, are a suitable candidate in many of these applications. One of them could be as a carrier structure for active substances, which could be applied to fields such as skin care and drug delivery. With the encapsulation of vitamins into diatom frustules, controlled release of the vitamin to the skin could be observed. With this practice, vitamin could be absorbed over a longer time period into the epidermis, giving more promising effects with skin care products including lotions, creams, serums, gels, etc. Moreover, the frustule itself can provide moisture and protection from detrimental ultraviolet radiation (280-400 nm). To examine whether vitamin encapsulation was successful, rhodamine encapsulation and release were first confirmed by using fluorescent microscopy and / or UV-Vis. Various diatom species were tested where the pennate *Craspedostaurus* sp. and the centric *Melosira* sp. were the most successful. To observe which diatom might be a potential best candidate, selected species were further inspected using SEM (Scanning Electron Microscopy), BET (Brunauer-Emmett-Teller) analysis and PCD (Particle Charge Detector) techniques to further study the frustule morphology, the surface area and particle charge. Further studies need to be conducted to check the encapsulation effectiveness for other vitamins, actives, proteins, drugs, or antibiotics, and more specifically controlled, targeted release for e.g. pharmaceutical implementation.

#### A sustainable approach for the seaweed use in agriculture

Choulot, M.<sup>1,2,3</sup>\*, Le Guillard, C.<sup>1</sup>, Michalak, I.<sup>2</sup> & Bourgougnon, N.<sup>3</sup>

<sup>1</sup>Agro Innovation International, Timac Agro, Plant Nutrition Department, Saint-Malo, France
 <sup>2</sup>Wroclaw University of Science and Technology, Wroclaw, Poland
 <sup>3</sup>Université de Bretagne Sud, EMR CNRS 6076, LBCM, IUEM, Vannes, France
 \*manon.choulot@univ-ubs.fr

For centuries, raw seaweeds have been spread in fields to improve soil properties and crops yield. Over last thirty years, the use of seaweed-based extracts has been gaining more and more attention in a global context where the agriculture is waiting for more efficient practices, environment-friendly solutions for a smooth and just transition. One of the solutions to mitigate abiotic stress are biostimulants. According to some projections, the European demand for seaweed-based biostimulants could be worth up to  $\in 1.8$ billion in 2030, this trend will probably continue in an EU committing to be climate-neutral in 2050.

In comparison with raw biomass, seaweed extracts are more concentrated in bioactive compounds such as macro/micro elements, peptides, polysaccharides, and targeted metabolites. Commonly, seaweed extracts used for agriculture purposes are obtained by water extraction at different pH and temperatures. However, these conventional methods are most often time consuming, with low selectivity and extraction yields and do not allow the preservation of the most sensitive compounds. In the last ten years, some tailored extraction processes using microwave, ultrasound and enzyme have demonstrated their advantages in obtaining enriched seaweed extracts.

In this work, we decided to apply one of these eco-friendly processes in a biorefinery approach to give added value to seaweed as a whole: (1) water-soluble extracts used as plant biostimulants or biofertilizers, and (2) post-extraction residues as biosorbent. Our process, which is designed to be sustainable and selective, was applied to three seaweeds selected as representatives of red, brown and green species.

### Mycosporine-like amino-acids from macroalgae and cyanobacteria sampled in French Polynesia

Connan, S.<sup>1\*</sup>, Lajili, S.<sup>1</sup>, Burel, L.<sup>1</sup>, Dronne, R.<sup>1</sup> & Zubia, M.<sup>2</sup>

<sup>1</sup>Univ Brest, CNRS, IRD, Ifremer, LEMAR, F-29280 Plouzane, France <sup>2</sup>University of French Polynesia, BP6570, 98702 Faa'a, Tahiti, French Polynesia \*solene.connan@univ-brest.fr In order to face environmental constraints, algae develop several defense mechanisms, including the avoidance or production of biomolecules. Thus, to protect themselves from too much light or certain harmful wavelengths such as UV radiation, algae produce photoprotective compounds that will attenuate the radiation reaching their photosynthetic apparatus. These compounds of different natures depending on the algae allow them to protect themselves, performing a passive and specific absorption of radiation, and dissipating the excess energy without inducing photochemical degradation. Our study focused on mycosporine-like amino acids (MAAs) from tropical algal species in order to find a new way of valorization in cosmetic Polynesian products.

The composition of MAAs of eight species of cyanobacteria and six species of red seaweeds from French Polynesia has been analyzed. These algae were sampled in five different islands (Tahiti, Moorea, Mangareva, Tubuai and Tahaa) and the spatio-temporal variability of MAAs composition has been studied for one red macroalgal species *Amansia rhodantha* in Tahiti. HPLC analysis of the samples identified a total of 21 MAAs or molecules absorbing between 310 and 360 nm. Among Cyanobacteria, *Hydrocoleum majus* contained the highest amount of MAAs and the highest diversity (9 MAAs). Among the red seaweeds, *Acanthophora spicifera, Amansia rhodantha* and *Gracilaria* sp. contained the highest MAAs level and the first two species had the highest MAAs diversity (9 MAAs). The spatio-temporal study of *A. rhodantha* showed strong inter-site and annual variations. To conclude, some Polynesian algae show great MAAs content and are present in sufficient biomass, such as *A. rhodantha* which is invasive, that could allow their potential valorization.

### Experimental design to optimize Bold's Basal Medium to increase biomass and pigments production in three microalgae species

Corrêa, P.S.<sup>1\*</sup> & Caetano, N.S.<sup>1,2,3</sup>

<sup>1</sup>CIETI, Center for Innovation in Engineering and Industrial Technology, School of Engineering, Polytechnic Institute of Porto, Portugal

<sup>2</sup>LEPABE, Laboratory for Process Engineering, Environment, Biotechnology and Energy, University of Porto, Portugal

<sup>3</sup>*ALiCE, Associate Laboratory in Chemical Engineering, University of Porto, Portugal* \*prisa@isep.ipp.pt

The great biotechnological potential of microalgae has aroused the interest of several industrial sectors. With biomasses rich in pigments, lipids, proteins and various other compounds, these microorganisms are a potential source of bioactive molecules for multiple applications. Chromochloris zofingiensis, for instance, is an interesting source of carotenoids, specially astaxanthin, Koliella longiseta is mainly reported as a rich source of eicosapentaenoic acid (EPA,  $\omega$ -3) and arachidonic acid (ARA,  $\omega$ -6), while Schizomeris leibleinii is mainly studied for wastewater treatment. The imposition of abiotic stress by changing culture conditions (e.g. nutrient concentration in the culture medium, temperature, light irradiance, among others) directly influences growth and is able to modulate biomass composition. An experimental design was proposed to evaluate the effect of salinity and concentration of nitrate, ferrous sulphate and phosphate on growth and production of carotenoids and chlorophylls in these species. Higher salinity and ferrous sulphate concentration increased the final biomass concentration almost threefold in C. zofingiensis and twofold in S. leibleinii when compared to their controls. Meanwhile, K. longiseta was able to double its biomass concentration only by changing nitrate concentration in the medium. Regarding pigment production, higher carotenoids content was found in C. zofingiensis (3.16 mg·g<sup>-1</sup>) cultivated in unmodified Bold's basal medium (BBM) and S. leibleinii (3.09 mg·g<sup>-1</sup>) cultivated under nitrogen limitation and increased salinity and ferrous sulphate concentration. Finally, higher chlorophyll content was observed in K. longiseta (17.77 mg $\cdot$ g<sup>-1</sup>) cultivated at higher ferrous sulphate concentration and *C. zofingiensis* (16.24 mg $\cdot$ g<sup>-1</sup>) cultivated in absence of phosphate and higher ferrous sulphate concentration.

# The brown macro-alga *Bifurcaria bifurcata*: an opportunity for the control of the parasitic nematode *Heligmosomoides polygyrus bakeri* in animal production

Miclon, M.<sup>1</sup>, Courtot, E.<sup>1</sup>, Guégnard, F.<sup>1</sup>, Lenhof, O.<sup>1</sup>, Boudesocque-Delaye, L.<sup>2</sup>, Matard-Mann, M.<sup>3</sup>, Collén, P.N.<sup>3</sup>, Davalle-Corre, K.<sup>3\*</sup>, Castagnone-Sereno, P.<sup>4</sup> & Neveu, C.<sup>1</sup>

<sup>1</sup>INRAE, Université de Tours, ISP, Nouzilly, France <sup>2</sup>EA 7502 Synthèse et Isolement de Molécules BioActives (SIMBA), Faculté de Pharmacie, Université de 8 Tours, 31 Avenue Monge, 37200 Tours, France <sup>3</sup>Olmix S.A., ZA du Haut du Bois, F-56580, Bréhan, France <sup>4</sup>INRAE, Université Côte d'Azur, CNRS, ISA, Sophia Antipolis, France \* karel.davalle-corre@olmix.com

The control of parasitic nematodes of livestock mainly relies on widespread use of anthelmintics, which has led to the development of resistance. In this context, the bioactive compounds from macro-algae offer attractive perspectives for the development of new solutions for gastro-intestinal parasites control. In the present study, we have investigated the potential anthelmintic activity of aqueous extracts from tree different seaweeds: *Bifurcaria bifurcata*, *Grateloupia turuturu* and *Osmundea pinnatifida*. The anthelmintic activity of the algae was determined on the murine parasite *Heligmosomoides polygyrus bakeri*, and a set of complementary in vitro tests: larval development assays, egg hatching tests as well as nematicidal activity assays on larvae and adults. Aqueous extracts from *B. bifurcata* revealed the best activity with a completely inhibited larval development at 5g/L and a IC50 value at 0.7g/L.

In addition, fractionation of the aqueous extracts with non polar solvents has been performed in order to identify the group of active molecules responsible for the anthelmintic activity. Heptane and ethyl acetate sub-fractions demonstrated the highest anthelminthic potential.

### Novel 'Biofilm PSBR' (Porous substrate photobioreactor) for benthic diatom cultivation

Arnaldo, M.D.G.<sup>1</sup>, Dehel Gamage, N.D<sup>1\*</sup>, Mossion, A.<sup>1</sup>, Wielgosz-Collin, G.<sup>1</sup>, Lebeau, T<sup>2</sup> & Méléder, V.<sup>1</sup>

<sup>1</sup>Institute of Substances and Organisms of the Sea (ISOMer), Nantes Université, France <sup>2</sup>Laboratory of Planetology and Geosciences, Nantes Université, France \*nadeeshani.dehel-gamage@etu.univ-nantes.fr

Benthic diatoms are the most representative photosynthetic microalgae group of the microphytobenthic community in shallow marine environments. They produce copious amounts of lipids as metabolites which can be used in various biotechnological applications of pharmaceutical, nutraceutical, food, cosmetics, and biofuel industries. Over the past 6 years, researchers of ISOMer laboratory have been focusing on potential valorization of marine benthic diatoms, in particular lipids. Thanks to the Nantes Culture Collection (NCC), Nantes Université, five benthic diatom species: *Amphora* sp., *Nitzschia* sp.,

*Nitzschia alexandrina*, *Opephora* sp., and *Staurosira* sp. have been identified as prospective candidates for lipid based biotechnological applications.

However, growth and lipid optimization of some oleaginous benthic diatoms; *Amphora* sp., *Opephora* sp. in suspension culture systems like airlift photobioreactor have become a challenge in respect to their sedentary lifestyle. Indeed, it has been demonstrated that some species of benthic diatoms grow when agitated while others produce higher biomass productivity and lipid rate when they are grown in undisturbed conditions. Thus, it is crucial to investigate the potential of biofilm-based cultivation system as an alternative to the currently prevailing suspension systems. In addition to respecting the physiology of the benthic species, this way of culturing consumes less water and makes it easy to harvest the biomass by scraping. That makes it more ecologically and economically sustainable.

To meet this challenge, our lab has developed a laboratory scale "biofilm photobioreactor" which gives a positive result in both growth and metabolite production, in particular lipids of benthic diatoms. The design of this PSBR, and the first results are presented in this poster.

### Seaweed-derived carbohydrates as a barley bio elicitor against infection with the fungus *Ramularia collo-cygni*

Delpont, W.\* & Popper, Z.A.

Botany and Plant Science, School of Natural Science, College of Science and Engineering, University of Galway, Galway, Ireland

\*w.delpont1@universityofgalway.ie

Barley, *Hordeum vulgare*, is a cereal grain mainly used for animal fodder, beer production and human consumption. In 2021, around 49 million hectares were cultivated for barley worldwide, with production reaching almost 147 million tonnes. Europe is the major barley producer with 61.7% of the total global production. However, *Ramularia collo-cygni*, the causative agent of Ramularia Leaf Spot (RLS) in barley can result in ~30% loss in yield and decreased grain quality. Climate change impacts in combination with recent legislation have led to increased incidence of many plant pathogens and in the case of RLS there are no effective available pesticides. There is therefore significant demand for effective, environmentally friendly, and sustainable alternatives to control RLS.

For this reason, the bioactive potential of different seaweed polysaccharides to protect plants against RLS is investigated. The extracted carbohydrates are investigated for their ability to a) stimulate the barley immune system and b) have direct antifungal effects. Several plant and seaweed-cell-wall-derived oligosaccharides have previously been shown to stimulate the plant immune system. We extracted polysaccharides from a range of brown, red, and green seaweeds using ethanol, and enzyme-assisted, methods. In order to test their elicitation effects, the different seaweed extracts were sprayed on three weeks aged barley plants two days prior to fungal inoculation. The first noticeable symptoms were observed 8-10 days after inoculation and an infection score was recorded for 4 weeks after infection. The results are expected to support efforts to develop sustainable, novel, broad-spectrum, plant protection products.

#### Algal inks, the future sustainable alternative for labelling

Field, J.<sup>1\*</sup>, Albers, S.<sup>2</sup>, Allen, R.<sup>1</sup>, Bury, H.<sup>1</sup>, Drysdale, C.<sup>1</sup>, Green, D.<sup>1</sup>, Lafferty, S.<sup>1</sup>, MacKechnie, K.<sup>1</sup>, Rad-Menéndez, C.<sup>1</sup>, Ross, M.<sup>1</sup>, Rowell, J.<sup>1</sup>, Saxon, R.<sup>1</sup>, Stanley, M.<sup>1</sup>, Thomas, N.<sup>1</sup> & Davey, M.P.<sup>1</sup>

<sup>1</sup>Culture Collection of Algae and Protozoa (CCAP) / Scottish Association for Marine science (SAMS), Oban, PA371QA, Scotland, UK <sup>2</sup>Living Ink Technologies, 601 1 Street, Berthoud, Colorado, 80513, USA \*joanne.field@sams.ac.uk

Algal derived inks are receiving increasing attention as sustainable replacements for petroleum-based or inorganic inks. Certain food and drinks companies strive to increase their sustainability credentials through using naturally occurring, biodegradable products in their packaging and labelling. Therefore, there is an urgent demand to advance the technology readiness level and make available pigments from algal sources. A working consortium formed between the Scottish Association for Marine Science (SAMS), Culture Collection of Algae and Protozoa (CCAP), and Living Ink Technologies (the leading US company in algal inks) have been engaged in projects, supported by Algae-UK and the European Institute of Innovation & Technology (EIT), to expand the portfolio of algal colours that Living Ink can use.

Across these collaborative projects, several algal and cyanobacterial strains were scaled-up and harvested using CCAP facilities. The stability of the pigments from these strains were tested under various pH and temperature conditions, representative of those experienced during the printing process. The raw biomass material underwent in-house testing at the Living Ink Technologies and processed for printing of newly designed labelling products. Investigating and applying methods for growing and harvesting algal biomass and investigating impacts of process production for the suitability of inks are necessary steps in the endeavour to produce carbon negative inks and increase the sustainability of the printing industry.

## Biochemical comparison between two populations of *Sargassum muticum*, introduced in the Pacific coast of Mexico and Atlantic coast of Brittany (France)

Gauyat, C.<sup>1,2,3</sup>\*, Hakoun, E.<sup>1</sup>, Serviere-Zaragora, E.<sup>2</sup>, Reyes, A.<sup>2</sup>, Sandoval, J.<sup>3</sup>, Connan, S.<sup>1</sup>, Le Grand, F.<sup>1</sup>, Kraffe, E.<sup>1</sup> & Stiger-Pouvreau, V.<sup>1</sup>

<sup>1</sup>IRD, Univ Brest, CNRS, Ifremer, LEMAR, F-29280 Plouzané, France, France <sup>2</sup>Centro de Investigaciones Biológicas del Noroeste (CIBNOR). Calle IPN # 195. La Paz, Baja California Sur. 23096, Mexico <sup>3</sup>Centro de Investigacion Científica y de Education Superior de Ensenada (CICESE). Carretera Ensenada-Tijuana No. 3918, Zona Playitas, C.P. 22860, Ensenada, B.C. Mexico

\*Camberra.Gauyat@univ-brest.fr

Since the 1970s, the European and Pacific coasts of respectively France and Mexico have experienced a significant and highly invasion of a benthic brown macroalga. *Sargassum muticum*, which can reach up to 10 metres in length, is the cause of brown tides and causes numerous tourists, economic and environmental problems in invaded countries. One solution is the use of high biomasses in industrial sectors. Numerous studies have demonstrated the potential (antioxidant, anti-inflammatory and anti-microbial activities among others) of *S. muticum* in Europe due to its high levels of secondary metabolites. However, there is a lack of data on the biochemical composition of *S. muticum* from the Pacific coast of Mexico to propose valorization pathways due to the difficult access to the sampling areas. Fresh Material of both *S. muticum* from France and North of Baja California were collected respectively in April and June 2022 at the beach of "Petit Dellec" (France) and "Bahia Todos Santos" (BCN, Mexico), before the reproductive period of the species. The proximate composition was

determined including crude and semi-purified fraction of phenolic compounds, crude proteins, carbohydrates, mannitol, free amino acids, lipids and pigments. This study presents this comparison which is discussed in regard to the introductive status of the species.

# Reconstruction and enrichment of the genome-scale metabolic network of the Fucale *Ascophyllum nodosum* and its microbiota with bioinformatics methods

Hamon-Giraud, P.<sup>1</sup>\*, Got, J.<sup>1</sup>, Belcour, A.<sup>1</sup>, Markov, G.<sup>2</sup>, Dittami, S.<sup>2</sup>, Rousseau, C.<sup>2</sup>, Cock, M.<sup>2</sup>, Potin, P.<sup>2</sup> & Siegel, A.<sup>1</sup>

<sup>1</sup>Univ Rennes, Inria, CNRS, Dyliss, IRISA, F-35000 Rennes, France <sup>2</sup>Sorbonne Université, CNRS, Integrative Biology of Marine Models (LBI2M), Station Biologique de Roscoff (SBR), 29680 Roscoff, France \*pauline.hamon-giraud@irisa.fr

Ascophyllum nodosum is a brown alga of the order Fucales abundant on the coast of Brittany. It has industrial applications due, in part, to its biostimulant properties on plants. It is yet undetermined whether those properties come from the alga itself, its microbiota, or the cooperation of both. The reconstruction and analysis of genome-scale metabolic networks (GSMNs) of the alga as well as all associated bacterial and fungal strains provide an approach to address this question.

Here we present the reconstruction and the curation-based biological knowledge of the GSMN of the host *A. nodosum*. Using the internally developed bioinformatics tool AuCoMe, we propagated the annotations of well-assembled and annotated brown algal models to the network of *A. nodosum*. Then, we developed precise manual and automatic curation procedures based on the Meneco gap-filling tool to ensure network functionality. The resulting network presently contains 2296 metabolites and 3039 biochemical reactions. The network predicts the production of 895 compounds through chains of reactions, starting from a list of 38 metabolites describing the sea water composition, each reaction's enzyme being supported by a gene sequence in the species genome. Fifty-five of these compounds are part of the 58 compounds we used to define the algal biomass.

The network will be further refined by adding the fungal partners and estimating bacterial GSMNs based on both metabarcoding data and genomes. All these networks will allow determining which metabolic pathways allow the production of metabolites of interest and which minimal holobiont community is sufficient to produce them.

#### Microalgal Ecotoxicological Testing: A Lipidomic Approach

Hartnell, D.M.\*, Cano Cejas, I., & Maskrey, B.H.

Centre for Environment, Fisheries and Aquaculture Science (Cefas), Barrack Road, Weymouth DT4 8UB, UK \*David.Hartnell@cefas,gov.uk

Phytoplankton, both eukaryotic algae and cyanobacteria, sustain our aquatic ecosystems and are often the first trophic level of biomagnification of any toxic compounds released into the environment. These compounds are highly diverse and can include persistent compounds (metals, PAHs, POPs etc.), hormones or pharmaceuticals, which can present long-term detrimental effects on aquatic organisms, ecosystems, and the services they provide. Many microalgae are used as test organisms in ecotoxicological challenges, which are evaluated by growth inhibition (mortality), but tests provide little information about cell physiology and the mechanisms of action of toxic compounds. Lipid profiles are diverse, amounting to over a thousand compounds which are key constituents of cells, maintaining cellular processes, as well as being involved in cellular signalling. In this study we applied a lipidomic approach utilising stat-of-the-art untargeted high-resolution mass spectrometry (HRMS) and using multivariate statistics were able to distinguish between, environment, domain, order, family, and genus in algal laboratory reference strains. Furthermore, two freshwater algal strains (*Microcystis* and *Chlorella*) were challenged with reactive oxygen species & superoxide generators, and HRMS & flow cytometry were applied to qualify and quantify responses on the algal lipidome.

The long-term aims of this study are to provide a valuable toolbox for toxicological testing, refining our understanding of how toxic compounds affect primary production in aquatic ecosystems. This is in-line with international guidelines, where animal ecotoxicological testing should be reduced, refined, or replaced (the 3Rs).

### Physicochemical- and bioactive properties of acid preserved Alaria esculenta and Saccharina latissima during storage

Hrólfsdóttir, A.Þ. 1\*, Arason, S.<sup>1,2</sup>, Sveinsdóttir, H.I.<sup>1,2</sup> & Gudjonsdóttir, M.<sup>1,2</sup>

<sup>1</sup>University of Iceland, Iceland <sup>2</sup>Matis, Iceland \*athh9@hi.is

Seaweed used for food and feed applications today are generally minimally processed, where they are mainly dried or frozen to reduce deterioration of the biomass. In Europe, the harvesting time of cultivated seaweed is very short, or about one to two months each year. To ensure a year-round supply of cultivated seaweed biomass there is a need for novel processing and preservation methods.

Acid preservation is a well-known method to preserve food, where the aim is to reduce the pH below 4.5 to inhibit microbial growth. To evaluate the effectiveness of acid preservation as a preservation method for seaweed, a shelf-life experiment was conducted with the two most cultivated species of brown seaweed in Europe, *Saccharina latissima* and *Alaria esculenta*. The biomass was either treated with lactic- or citric acid and stored over approximately six-month period. Physicochemical (including proximate composition, total phenolic content (TPC), texture and pH), microbial- and antioxidant (ORAC, DPPH) analysis were performed during storage and sensory attributes of the preserved biomass examined to evaluate possible uses.

Results show that the proximate composition, color, pH, and texture of the acid-preserved seaweed is relatively stable throughout the storage time. However, a decrease was observed in TPC and antioxidant properties (assessed by DPPH) during storage of the acid preserved biomass. Meaning, the method might be good to stabilize the biomass for feed food and feed applications but not if intended for antioxidant purposes. However, the acid treated biomass might be suitable as an ingredient for value-added products.

# The impact of enhancing tolerance to Rose Bengal on growth, photosynthesis and lipid metabolism in *Nannochloropsis oceanica* under suboptimal conditions

Htet, H.Y.\*, Ben-Sheleg, A., Vonshak, A. & Khozin-Goldberg, I.

Microalgal Biotechnology Laboratory, The French Associates Institute for Agriculture and Biotechnology of Drylands, The Jacob Blaustein Institutes for Desert Research, Ben-Gurion University of the Negev, Sede Boqer Campus, Midreshet Ben-Gurion 8499000, Israel \*hsuyee@post.bgu.ac.il

The Nannochloropsis genus of microalgae, which includes N. oceanica, is widely explored in microalgal biotechnology for production of the omega-3 LC-PUFA, eicosapentaenoic acid. However, biomass and LC-PUFA production of microalgal cultures, especially in outdoor settings, are often hindered by stresses associated with oxidative stress conditions. To improve the tolerance of N. oceanica to oxidative stress, mutant strains, designated RB2 and RB113, were isolated based on the tolerance to Rose Bengal (RB), a photosensitizer of singlet oxygen. The RB strains were shown to exhibit enhanced photosynthetic capacity in excess light and oxygen environments compared to the WT strain. RB113 mutant carries a mutation in the 2-oxoisovalerate dehydrogenase E1 subunit of the mitochondrial branched-chain α-keto dehydrogenase complex, while RB2 has multiple gene variants in its genome. In the present work, we further investigated the response of RB mutants to challenging environments. Under high light (500 µmol photons m<sup>-2</sup> s<sup>-1</sup>) at 25°C, all strains exhibited a lag phase characterized by a drop in the maximum yield of PSII (Fv/Fm), a decrease in the percentage of EPA, associated with the transient formation of triacylglycerols (TAG) in lipid droplets. However, both RB strains showed enhanced photosynthetic efficiency and recovery of photosynthetic parameters. In particular, RB113 was superior in restoring EPA to pre-stress levels. The enhanced ability of RB113 to withstand and thrive under unfavorable conditions was observed during cultivation at 22°C. These conditions caused severe growth arrest, alterations in morphology and fatty acid composition, and persistent presence of lipid droplets. Through global proteomic analysis shared and strain-specific differentially abundant proteins were revealed. Notably, delta12 desaturase, betaine lipid synthase, certain mitochondrial proteins, and proteins important for the proper folding, and stability, appeared to be significantly increased in RB113, providing insights on the molecular mechanisms underlying the improved stress tolerance in RB113.

# Physiological responses of a tropical microalgae, *Tetraselmis sp.*, to UV-A stress in continuous culture: biomass, antioxidant activity, and photosynthetic efficiency

Isaia, A.<sup>1,2</sup>\*, Coulombier, N.<sup>3</sup>, Le Dean, L<sup>1</sup>, Meriot, V<sup>1,4</sup> & Jauffrais, T<sup>1</sup>

<sup>1</sup>Ifremer, IRD, Univ Nouvelle-Calédonie, Univ La Réunion, CNRS, UMR 9220 ENTROPIE, 101 Promenade Laroque, 98897, Nouméa, New Caledonia

<sup>2</sup>Univ Brest, CNRS, IRD, Ifremer, LEMAR, F-29280 Plouzané, France

<sup>3</sup>ADECAL Technopole, 1 bis rue Berthelot, 98846 Nouméa, New Caledonia;

<sup>4</sup>ISEA, EA7484, Université de Nouvelle Calédonie, Campus de Nouville, 98851, Nouméa, New Caledonia \*anna.isaia@univ-brest.fr

Microalgae are able to produce an array of valuable compounds with bioactive properties. Among them, natural antioxidant molecules from microalgae are getting more and more attention owing to their potential application in cosmetics, pharmaceuticals, and nutraceuticals. The production of such molecules can be oriented by abiotic factors such as light, nutrient, ultraviolet (UV), and pH.

In the perspective of optimizing antioxidant activity, we investigated the effect of UV-A radiation on a *Tetraselmis* sp., which is known to have a high growth rate, and has attracted interest owing to its antioxidant activity. Cultures were exposed to UV-A radiation in parallel to photosynthetically active radiation in photobioreactors operated in continuous. The exposure to UV-A affects both the photosynthetic and antioxidant activity of *Tetraselmis*. Short-term exposure to UV-A showed a negative effect on the maximal photosynthetic efficiency (Fv/Fm), more specifically on the electron transport

chain. However, a resilience of most physiological parameters was observed over the experiment (10 days) suggesting a photochemical adaption over long-term exposure. Nonetheless, the addition of UV-A decreased the antioxidant activity of the microalgal extract suggesting the use of antioxidant molecules to avoid irreversible damages. Finally, the best antioxidant activity never observed with a *Tetraselmis* was measured in cultures without UV addition, with an IC50 of  $2.87 \pm 0.24 \,\mu g \, mL^{-1}$  close to the reference compounds Trolox and  $\alpha$ -tocopherol.

Here, we confirmed the potential of *Tetraselmis* for antioxidant production when grow under optimal conditions, but we discourage the use of UV-A to enhance antioxidant activity with this species.

### Bioactivity screening of indigenous microalgae from freshwater for cosmeceutical application

Jin, C.\*, Ye, Z. & Lee, H-G.

Cell Factory Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Republic of Korea \*chunsik@kribb.re.kr

This research project aimed to screen natural pigments produced by indigenous microorganisms by isolating hundreds of microalgae from various freshwater sources in South Korea. A total of 108 candidates were selected based on their ability to survive abiotic stress. Phylogenetic classification was based on 18s rRNA, ITS, and rbcL genes, with 19% of the isolates identified as *Chlorella* sp. A microalgal ethyl acetate extract library was constructed, and secondary metabolites were profiled using LC/MS. The biological activities of the extracts were evaluated for their potential as cosmetics and medicine, including UV protection, whitening, and antioxidant properties, as well as a zebrafish gastrulation genotypic assay. Seven strains of *Chlorella* sp. with functional bio-activity were identified from approximately 20 strains. After optimizing phototrophic cultivation, these strains were further analyzed for their physiological and cosmeceutical properties. Among them, *Chlorella* sp. CV0051 exhibited the highest effect on skin whitening. Further identification of the active compounds and optimization of their production make these indigenous *Chlorella* sp. strains potential bioresource candidates for the cosmeceutical industry.

### Hydrocarbon continuous production and non-destructive extraction from the microalga *Botryococcus braunii*

Kemel, S.\*, Marchal, L., Goncalves, O. & Pruvost, J.

Nantes Université, Oniris, CNRS, GEPEA, UMR 6144, Saint-Nazaire, France \*samy.kemel@univ-nantes.fr

Oil is a largely used limited non-renewable resource subject to repetitive price increases. The development of new sustainable industrial processes to produce this resource is one of the possible solutions to help achieve carbon neutrality goals. *Botryococcus braunii* is a colonial microalga that has attracted significant attention in recent years due to its ability to produce hydrocarbons through photosynthesis. The main objective of my thesis is to develop an integrated process of hydrocarbon production and extraction. However, this microalga presents a slow growth and stores the hydrocarbons in its extracellular matrix, limiting production and extraction.

We propose to improve growth by optimizing the light absorbed by the cells in a thin-film photobioreactor. Moreover, hydrocarbon non-destructive extraction technics are being investigated to

keep the microalgae alive, saving resources and helping to overcome the low growth rate. The microalga has been successfully grown in a torus photobioreactor, and solvent free extraction processes using pressure have been tested out. The colony size distributions before and after extraction are very little altered, which constitutes a promising sign for cellular viability. Despite the low yield of extraction obtained (around 3%), repetitive extraction to match the hydrocarbon productivity is possible, allowing envisaging a continuous process of hydrocarbon production. Therefore, the impact of this process on the microalga viability will be studied, and the protocol will be transposed to thin-film photobioreactor to assess hydrocarbon productivity.

#### Differential growth of gut, skin and soil bacteria induced by compounds isolated from brown macroalgal species collected on Atlantic coasts, with potential application for industry

Kergosien, N.<sup>1,2</sup>\*, Brébion, J.<sup>1</sup>, Connan, S.<sup>2</sup>, Hennequart, F.<sup>1</sup> & Stiger-Pouvreau, V.<sup>2</sup>

<sup>1</sup>ALGAIA, R&D center, Saint-Lô, France <sup>2</sup>Univ Brest, CNRS, IRD, Ifremer, LEMAR, F-29280 Plouzane, France \*nolwenn.kergosien@algaia.com / nolwenn.kergosien@gmail.com

Gut and skin microbiota are major contributors of human well-being. They participate in the immune system development, prevent pathogens colonization, and promote tissue repair and barrier functions. Disbalances in the gut and skin microbiota often lead to various inflammatory disturbances. In parallel, soil microbiome is an important parameter for soil health and structure, as well as plant growth, as it improves nutrients recycling and water retention. Brown macroalgal extracts can modulate, *i.e.* promote and/or limit, bacterial growth. Considering the importance of balanced microbiome for human and soil health, the present study aims at finding extracts that promote beneficial and limit pathogenic bacteria growth. Crude extracts and purified fractions were obtained from six Phaeophycean specimens from the Atlantic Ocean (tropical holopelagic Sargassum natans I and VIII and S. fluitans III; temperate benthic Ascophyllum nodosum, Laminaria digitata and L. hyperborea) and studied for their effects on bacterial growth on eight bacterial strains (two beneficial and three pathogenic gut bacteria, one pathogenic skin bacteria and two beneficial soil bacteria). A mild acid extraction was used on the algal biomass to obtain crude extracts, from which the compounds were separated by tangential filtration, and alginate-rich fractions were obtained from the extraction residual biomass. The biochemical composition of crude extracts and fractions was characterized and compared to the bacterial assays. Synergies between compounds were studied through the variations in bacterial activities from crude extracts to purified fractions, to highlight the potential of Atlantic brown macroalgal species for soil quality and human wellness applications.

#### Microalgae-Based Integrated Biorefinery for Simultaneous Production of Commercially Valuable Carotenoids and Plastic Composites

Kim, H.-S.<sup>1,2</sup>

<sup>1</sup>Cell Factory Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Daejeon, South Korea 34141

<sup>2</sup>Department of Environmental Biotechnology, KRIBB School of Biotechnology, University of Science and Technology (UST), Daejeon, South Korea 34113

#### hkim@kribb.re.kr

Questions around the economic feasibility of microalgae-based products have remained not fully addressed as challenges from contamination in large-scale industrial cultivation systems to high energy costs associated with biomass harvesting and subsequent downstream processes prevail. Researchers and practitioners in the field are thus actively exploring "biorefinery" approaches, through which whole microalgal biomass is utilized in multiple commercialization routes. To this end, two ideas have been explored following the extraction of hydrophobic fractions of biomass, including commercially valuable carotenoids: (1) saccharification of residual "defatted" microalgal biomass, the process that could be eventually combined with the fermentation of other microbes (including microalgae) producing highvalue bioproducts (2) filler material application of left-over residual biomass in the fabrication of plastic composites. The results indicated that each of these approaches has a potential to bolster the overall economic outlook of microalgae-based biorefinery, even though a careful techno-economic assessment would be needed. Importantly, a preliminary evaluation of residual biomass recovered following defatting (i.e., solvent extraction) and saccharification suggested a further improvement in the mechanical properties of the resulting polymer-biomass composite, possibly because of an increase in the relatively proportion of proteinaceous compounds. Combined together, the results of preliminary investigations encourage the integration of biorefinery processes that do not mandate a substantial modification to existing industrial microalgae processors.

#### The effect of different light intensities and nitrogen concentrations on the interdependence of fucoxanthin, chlorophyll *a/c*-binding protein and fucoxanthin biosynthesis in *Phaeodactylum tricornutum*

Kim, S.M.<sup>1,2</sup>\*, Truong, T.Q.<sup>1,2</sup>, Song, D-G.<sup>3</sup> & Park, Y.J.<sup>1</sup>

<sup>1</sup>Smart Farm Research Center, KIST Gangneung Institute of natural Products, Republic of Korea <sup>2</sup>Division of Bio-Medical Science & Technology, Korea Institute of Science and Technology (KIST) School, University of Science and Technology, Republic of Korea <sup>3</sup>N to al Part of the Pa

<sup>3</sup>Natural Products Informatics Research Center, KIST Gangneung Institute of natural Products, Republic of Korea

\*kimsm@kist.re.kr

Fucoxanthin (FX) is a carotenoid that is most abundant in the chloroplasts of brown seaweeds and some microalgae. The microalga *Phaeodactylum tricornutum*, a member of Baccillariophyta, has been identified as a potential commercial source of FX. In *P. tricornutum*, FX is produced via carotenogenesis and forms the FX, chlorophyll *a/c*-binding protein (FCP) in the thylakoid membrane. Our studies consistently demonstrate that low light and replete nitrogen are favorable for FX accumulation in *P. tricornutum*. While light plays a critical role in regulating the biosynthesis of photosynthetic pigments, nitrogen is a major nutrient element in the growth medium. While low light and nitrogen both have a positive effect on FX accumulation, the mechanisms behind this have not been fully understood. Moreover, the correlation between FX accumulation and FCP biosynthesis under different conditions has not been completely elucidated. Therefore, the objective of this study is to investigate the effects of light intensity and nitrogen on FX biosynthesis by analyzing the interdependence of FX and FCP. *P. tricornutum* was grown under low (20 µmol photons/m<sup>2</sup>/s) and high light (200 µmol photons/m<sup>2</sup>/s) conditions and with standard F/2 medium and 10X nitrate-contained medium to investigate the effects of light and nitrogen. Cell growth and FX concentration were monitored daily, while SDS-PAGE coupled with LC-MS/MS was utilized to identify the compositions of FCP, and RNA sequencing was

carried out to select key genes involved in the carotenogenesis of FX and the synthesis of FCP protein. The findings suggest that FX biosynthesis is interdependently regulated with FCP biosynthesis under both light and nutrient conditions.

### BlueBio MINERVA: Exploring the potential of Irish macroalgae for sustainable valorisation with focus on *A.* nodosum and *S. latissima*

Lalegerie, F.\* & Stengel, D.B.

Botany and Plant Science, School of Natural Sciences, Ryan Institute for Environmental, Marine and Energy Research, University of Galway, Galway, Ireland \* fanny.lalegerie@universityofgalway.ie

Environmental fluctuations strongly impact macroalgal physiology and associated chemical composition through multiple, interactive effects making algal responses complex to assess and predict. The resulting natural variability needs to be considered from a valorisation perspective, to ensure a constant biomass production and composition for a sustainable exploitation of natural populations. In particular, the long-term consequences of climate change on commercial species and their subsequent valorisation are unclear. In this context, a cultivation experiment was conducted on Ascophyllum nodosum and Saccharina latissima (Phaeophyceae, Ochrophyta), to assess changes in their productivity and chemical composition in response to increasing temperature. Both species were collected in Galway Bay (Ireland), and cultivated at different temperatures for 3 weeks. Photosynthesis was measured by Pulse-Amplitude Modulation (PAM) fluorometry at different time intervals. Results highlight higher maximum quantum yield and photosynthetic efficiency after shortterm exposure to high temperature, and A. nodosum appeared to better acclimate to long-term high temperature conditions. Responses measured by chlorophyll fluorescence are compared with O2-evolution to evaluate implications of experimental approaches when assessing temperature effects. In parallel, a seasonal screening (i.e. phenolic content, pigment composition, antioxidant and antimicrobial activities) of 19 other Irish macroalgae was undertaken to assess the potential of valorisation of local biomass, to guide the exploration of additional seaweed resources in the future. This study represents part of the BlueBio Cofund project MINERVA ('Marine Innovation using Novel Enzymes for waste Reduction and Valorisation of Algal Biomass').

## BlueBio MINERVA: Unlocking the potential of macroalgae for a sustainable valorisation in BlueBioeconomy, using new processes and a biorefinery approach

Lalegerie, F.<sup>1\*</sup>, Tretiak, S.<sup>1</sup>, Jackson, S.<sup>2</sup>, Dobson, A.<sup>2</sup>, Hotchkiss, S.<sup>3</sup>, Jónsdóttir, R.<sup>4</sup>, Berglin, M.<sup>5</sup>, Popper, Z.A.<sup>1</sup> & Stengel, D.B.<sup>1</sup>

<sup>1</sup>Botany and Plant Science, School of Natural Sciences, Ryan Institute for Environmental, Marine and Energy Research, University of Galway, Galway, Ireland; <sup>2</sup>School of Microbiology, University College Cork, Cork, Ireland; <sup>3</sup>CyberColloids, Carrigaline, Co. Cork, Ireland; <sup>4</sup>Matís, Iceland and UNA Skincare, Iceland; <sup>5</sup>RISE, Sweden \*fanny.lalegerie@universityofgalway.ie Seaweeds represent an attractive and promising resource towards the realization of the European Circular Blue Bioeconomy as they synthetize a range of valuable compounds with multiple industrial applications. Nevertheless, further efforts are needed to develop new high value-added products in order to unlock the full economic potential of seaweeds which are still considered an under-exploited resource in Europe. In this regard, the BlueBio Cofund project MINERVA ('Marine Innovation using Novel Enzymes for waste Reduction and Valorisation of Algal Biomass') supports an efficient algal industry by developing new extraction processes and omics-based approaches, and considering biomass / application diversification in the extraction of algal bioactive compounds through a biorefinery strategy. Furthermore, MINERVA promotes a sustainable industry and exploitation of algal biomass, based on principles of waste reduction, the study of the effects of global change and the optimization of algal biomass. Joint efforts are conducted to develop targeted applications in food, aquaculture, cosmetics, and biomedicine industries, with partners from Ireland, Iceland and Sweden. The project has facilitated the optimization of extraction processes, with a particular emphasis on polysaccharides, proteins and phenolics. Bacterial isolates from brown algae exhibited cell-wall biodegradation potential which have been applied to further increase extraction efficiencies. Algal extracts/components have demonstrated antioxidant, antimicrobial, antifouling activities and/or particular physicochemical properties, which have been used in the formulation of antifouling paints for aquaculture, cosmetics products, and biomaterials at pilot scale. Additionally, market positioning based on technical functionality and benchmarking was conducted for incorporation of brown seaweed fibres in different food applications.

### Spatial variability of phlorotannins extracted from *Hormosira banksii* along a longitudinal gradient in Victoria (Australia)

Lam Kim, F.<sup>1,2\*</sup>, Stiger-Pouvreau, V.<sup>2</sup> & Bellgrove, A.<sup>1</sup>

<sup>1</sup>Deakin University, School of Life and Environmental Sciences, Centre for Integrative Ecology, Warrnambool Campus, Princes highway, Warrnambool, Victoria 3280, Australia <sup>2</sup>Laboratoire des Sciences de l'Environnement Marin (UMR 6539), Institut Universitaire Européen de la Mer, Université de Bretagne Occidentale, rue Dumont d'Urville, 29280 Plouzané, France \*fmlamkim@deakin.edu.au

In the harsh conditions of the intertidal zone, seaweeds must face numerous stresses such as desiccation, temperature, and UV radiation. In temperate waters of Australasia, a brown alga dominates the rocky shores: *Hormosira banksii*. In a place where UV radiation is among the highest in the world, the photoprotection of the habitat-forming fucoid, showing apparent resistance to light stress, was investigated. Photoprotective, and antioxidant, phlorotannins are known to have a significant role in brown algae. Nevertheless, their content is highly fluctuating.

To assess the variability of phlorotannins along a longitudinal gradient of temperature and UV index, low and high shore samples of *H. banksii* were collected from eight sites along Victoria coastline, divided into four regions (Southwest Victoria, Great Ocean Road, Mornington Peninsula and Port Phillip Bay). Total phenolic content (TPC) was assessed, and the antioxidant activity was estimated using DPPH radical-scavenging activity and compared with chemiluminescence measured by an innovative HPLC system tested on seaweed extracts for the first time.

A significant variability was observed between sites but was not correlated to temperature and UV index. In fact, this may result in the interaction of various factors including some that were not primarily considered, such as wave-exposure. Moreover, some sites revealed incredibly high phenolic contents (especially sites within the region Great Ocean Road) and related antioxidant activities. The high contents in phlorotannins discovered in this study suggest a real potential of *H. banksii* for commercial

applications but this also underlines the importance of understanding better what drives spatial variability.

### Enhancement of carotenogenesis and neutral lipid production by heterologouse overexprssion of orange protein in *Chlamydomnas*

Lee, H-G.\*, Lee, M-W., Jin, C.

Cell Factory Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Republic of Korea

\*trustin@kribb.re.kr

Microalgal carotenoids and lipids have recently become popular as potential ingredients in cosmetics, pharmaceuticals, and alternative energy sources. This study aimed to develop a microalgal strain capable of simultaneous production of carotenoids and triacylglycerol by heterologously expressing orange protein in *Chlamydomonas reinhardtii*. Two-stage cultivation with nitrogen starvation for two days was used to induce triacylglycerol production in the transformants. During nitrogen deprivation, the effects of endogenous abscisic acid and intracellular reactive oxygen species on triacylglycerol biosynthesis were investigated. The results showed that the transformants had higher levels of total carotenoids and  $\beta$ -carotene compared to the wild-type strain under nitrogen deficiency. Additionally, the transformants exhibited significant increases in triacylglycerol accumulation along with elevated levels of abscisic acid and reactive oxygen species. These findings suggest that the transformants have the potential to serve as a microalgal feedstock for the simultaneous production of carotenoids and triacylglycerol.

### Characterization of bioactive oligosaccharides isolated from the red algae Solieria chordalis

Lesgourgues, M.<sup>1,2\*</sup>, Latire, T.<sup>1,2</sup>, Bourgougnon, N.<sup>1</sup> & Bedoux, G.<sup>1</sup>

<sup>1</sup>Laboratoire de Biotechnologie et Chimie Marines, Université Bretagne Sud, EMR 6076, IUEM, 56000 Vannes, France.

<sup>2</sup>Université Catholique de l'Ouest Bretagne Nord, 22200 Guingamp, France

\*mathilde.lesgourgues@univ-ubs.fr

In Brittany, approximately 10,000T of the red macroalgae, *Solieria chordalis*, beach cast every year. This under-exploited biomass, rich in bioactive compounds, has been explored in the framework of numerous research projects conducted by our laboratory. Extracts of this seaweed have shown interesting biological activities for cosmetic applications.

The objective of this work is the production of oligosaccharides with biological activities from the parietal polysaccharides of *S. chordalis* collected in Saint Gildas de Rhuys (France). The project is organized in two steps: the first concerns the production of oligosaccharides by depolymerization of carrageenans and their characterization. The second step aims to evaluate the biological properties on different types of cutaneous cells (keratinocytes, fibroblasts).

The preliminary extraction of carrageenans leads to yields ranging from 6 to 22% depending on the parameters monitored and the number of extractions. After biochemical characterizations, the parietal polysaccharides (PPS) were studied by different chromatographic (HPSEC and HPAEC) and spectroscopic (IR-TF) techniques. Depolymerization by the action of hydrogen peroxide or ultrasounds reduce the mass of PPSs initially greater than 1 MDa to molecules with masses between 200kDa and

15kDa. Furthermore, the monosaccharide compositions revealed the majority presence of glucose and galactose as well as other minority monosaccharides.

After presentation of the viability and cytotoxicity results, the methodology for studying the properties of the extracts on the components of the dermal extracellular matrix will be described with particular attention paid to the evaluation of anti-inflammatory, anti-ageing, hydration and skin homeostasis activities.

### Sporelings and growth of the marine red alga, *Gelidium elegans* (Rhodophyta), from Northeast Taiwan

Lin, S.-M.\*, Chiou, T.-H., Chiou, Y.-S. & Lin, L.-C.

Institure of Marine Biology, National Taiwan Ocean University, Taiwan, R.O.C. \*LINSM@NTOU.EDU.TW

The Species of *Gelidium* are economically important agar-containing marine red algae. *Gelidium elegans* is widely distributed in the northwestern Pacific Ocean. In recent years, wild populations of *G. elegans* have been gradually declined in Northeast Taiwan. In this study, we tested two sets of culture temperatures (18°C and 26°C) to understand the sporelings and growth of *G. elegans* under global warming. Several tetrasporic thalli bearing mature tetrasporangia were collected from the field in the spring of 2019-2020. Two experiments were performed to determine whether or not the seedlings germinated from tetraspores could survive under temperature conditions in early spring (18°C) and early summer (26°C) in northern Taiwan. Most tetraspores germinated into seedlings at 18°C and 26°C, but the seedlings at 26°C ceased to grow after a few months of cultivation. On the other hand, the juveniles at 18°C grew into adult sizes (more than 10 cm high) after more than a year of cultivation in the lab. We will discuss the main factors responsible for the decline of *G. elegans* in Taiwan. Outdoor cultivation experiments will be presented during the oral presentation.

### Real time, non-invasive monitoring of microalgae biofilm-based cultivation systems using reflectance spectroscopy

Morgado, D.<sup>1\*</sup>, Fanesi, A.<sup>1</sup>, Martin, T.<sup>1</sup>, Tebbani, S.<sup>2</sup>, Bernard, O.<sup>3</sup> & Lopes, F.<sup>1</sup>

<sup>1</sup>LGPM, CentraleSupélec, Université Paris-Saclay, Gif-sur-Yvette France <sup>2</sup>L2S, CentraleSupélec, Université Paris-Saclay, CNRS, Gif-sur-Yvette, France <sup>3</sup>Biocore, INRIA, LOV, INRAE, Sophia-Antipolis, France \*david.morgado-pereira@centralesupelec.fr

Microalgae Biofilm-based Cultivation Systems (MBCS) are emerging as a promising solution for microalgae production, offering increased productivity, simplified harvesting, and lower operating costs. However, efficient monitoring routines for MBCS are still lacking, and current approaches involve invasive and destructive sampling, which emphasizes the need for non-invasive online monitoring tools. In this study, we introduce for the first time a novel online protocol that utilizes reflectance spectroscopy to monitor MBCS. This remote sensing technology, widely employed in smart agriculture, relies on reflectance indices (RIs) obtained from the reflected light in the visible to near-infrared (Vis-NIR) range, providing a non-destructive approach for biofilm monitoring.

This approach was validated using a biofilm-based rotating system to cultivate *Haematococcus pluvialis*. This chlorophyceae was grown as a biofilm on cotton supports, for producing astaxanthin, a high-value carotenoid. We quantified biomass, chlorophyll, and astaxanthin dynamics under four light

and two nutrient conditions and collected reflectance spectra on the same samples to identify the spectral bands that correlated the most with these biofilm parameters.

Based on these correlations, we developed robust linear models (R2 > 0.90, nRMSE < 10%) capable of predicting biomass and astaxanthin areal density (g/m2), as well as astaxanthin and chlorophyll content (g/gDW), regardless of the cultivation conditions.

Our pioneering study demonstrates the feasibility of reflectance spectroscopy as a realtime, non-invasive monitoring tool to improve the operation and efficiency of MBCS. The application of this technology to other microalgae strains producing high-value compounds has the potential to boost the field of biofilm-based systems.

## Potential of Djiboutian abundant macroalgae as food and feed ingredients

Nour, M.<sup>1,2</sup>\*, Petek, S.<sup>1</sup>, Daher, A.<sup>2</sup>, Connan, S.<sup>1</sup>, Marchand, L.<sup>1</sup>, Waeles, M.<sup>1</sup> & Stiger-Pouvreau, V.<sup>1</sup>

<sup>1</sup>Univ Brest, IRD, CNRS, Ifremer, LEMAR, F-29280 Plouzane, France <sup>2</sup>Centre d'Étude et de Recherche de Djibouti, Institut des Sciences de la Vie ISV, Laboratoire de biologie marine, Route de l'aéroport BP 486, Djibouti, Republic of Djibouti \*moustapha.nouribrahim@univ-brest.fr

Marine abundant macroalgae from Djibouti (Gulf of Tadjourah) were collected in different stations from which the biochemical composition together with trace element and heavy metals were determined. Phenolic contents varied from  $8.88 \pm 0.13$  to  $0.45 \pm 0.06$  mg PGE/g DW, for respectively Padina pavonica and Hypnea sp. The most active antioxidant activities, i.e.  $0.08 \pm 0.04$  mg/mL with DPPH and  $0.035 \pm 0.001$  mg/mL with FRAP tests, were recorded in the *Padina pavonica* collected in Khorambado. Protein contents varied from 5.89  $\pm$  0.62 for the Hypnea sp. to 18.62  $\pm$  0.19% in Padina *pavonica*. For carbohydrates, contents varied from  $14.42 \pm 0.33$  % DW in the brown Sargassum sp. to  $38.36 \pm 1.09$  % in Hypnea sp., mannitol varied from  $10.12 \pm 3.78$  in Sargassum sp. to  $39.78 \pm 1.87$  in *Padina pavonica* from Khor-ambado. Amino acids ranged from  $10.41 \pm 0.26$  mg/g DW to  $25.31 \pm 1.71$ mg/g in the brown Turbinaria ornata. Regarding pigments, total chlorophyll a and c contents were maximum in *Padina pavonica*  $(5.21 \pm 0.10 \text{ mg/g DW})$  and in Sargassum sp.  $(1.37 \pm 0.22 \text{ mg/g DW})$ , their minimum contents were observed at  $0.068 \pm 0.01$  mg/g DW in T. ornata and  $0.20 \pm 0.13$  mg/g DW in S. binderi respectively, while Fucoxanthin content was highest in Sargassum sp.  $(2.09 \pm$ 0.33mg/g DW). The contents of minerals and trace elements were measured and gave maximum values for Padina pavonica whatever the harvesting location. Since these seaweeds are abundant in Djiboutian coastal waters, their biochemical composition and associated antioxidant activities, have made Djiboutian abundant seaweeds, promising candidates for nutritional, pharmaceutical, and medicinal applications.

# Assessment of the biochemical parameters of *Arthrospira platensis* cultivated semi-continuously using three nutrient replenishment media for industrial production

Nunes, N.\*<sup>1,2</sup>, Fernandes, T.<sup>3</sup>, Fernandes, I.<sup>3</sup>, Jardim, B.<sup>1</sup>, Barros, A.I.R.N.A.<sup>2</sup> & Pinheiro de Carvalho, M.Â.A.<sup>1,2,4</sup>

<sup>1</sup>ISOPlexis Centre Sustainable Agriculture and Food Technology, University of Madeira. Campus da Penteada, 9020-105 Funchal, Portugal

<sup>2</sup>Centre for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB), University of Trás-os-Montes and Alto Douro, 5000-801 Vila Real, Portugal

<sup>3</sup>Phytoalgae Lda., Caminho do Pedregal, Nº 32, 9350-060, Ribeira Brava, Portugal

<sup>4</sup>Faculty of Life Sciences, University of Madeira, Campus da Penteada, 9020-105 Funchal, Portugal.

\*nuno.nunes@staff.uma.pt

The blue-green cyanobacteria Arthrospira platensis, commonly known as Spirulina, due to its physical appearance, is progressively gaining worldwide visibility, due to conscious shifting in consuming alternative and vegan food, highly nutritive. In this work, three different feeding media were tested after initially cultivating these microorganisms in Zarrouk medium. Biomass was harvested at the end of three semi-continuous cycles at days 6, 12 and 18. Nutrients were reintegrated proportionally to the collected biomass using three different feeding media. Protein content started in the first cycle with 15 to 32 g/100g dw reaching at the end of the third cycle with 41 and 56 g/100g dw, respectively. It was observed that lipids concentration decreased along the cycles, starting at 6 and 8 g/100g dw and lowering to 4 and 5 g/100g dw at the end of the third cycle, respectively. Total minerals also decreased along the cycles, starting with 12 and 18 g/100g dw and decreasing till 9 and 10 g/100g dw, respectively. Chlorophyll a started between 4 and 6 mg/100g dw and at the end of the third cycle these values were a little higher, reaching 6 and 7 mg/100g dw, respectively. Total carotenoids concentration started with values around 0.8 mg/100g dw and increased to 1.8 mg/100g dw at the end of the third cycle. This work is extremely relevant, since it demonstrates that A. *platensis* can be cultivated for a long-term without damaging its biochemical traits using recycled media. Further studies targeting the optimization of A. platensis feeding media could be developed to locally produce A. plantensis at an industrial scale with reduced maintenance. This work is incorporated into the PhytoNutre project, which is promoted by the company Phytoalgae in partnership with the University of Madeira, with EEA Grants funding (PTINNOVATION-0118).

## Microalgal-based systems for sustainable wastewater upcycling, bioprocess optimization by mechanical stress

Ortenzi, F.<sup>1,2\*</sup>, Savio, S.<sup>1</sup>, Montereali, F.<sup>3</sup>, Mancini, T.<sup>3</sup>, Contaldo, A.<sup>3</sup>, Buccarello, L.<sup>3</sup>, Di Cave, A.<sup>1,2</sup>, Rugnini, L.<sup>1</sup>, Antonaroli, S.<sup>4</sup>, Morozzo Della Rocca, B.<sup>5</sup>, Bruno, L.<sup>1†</sup> & Congestri, R.<sup>1</sup>

<sup>1</sup>Laboratory of Biology of Algae, Department of Biology, University of Rome 'Tor Vergata', Via della Ricerca scientifica 00133 Rome, Italy

<sup>2</sup>*PhD Program in Evolutionary Biology and Ecology, Department of Biology, University of Rome 'Tor Vergata', Via della Ricerca scientifica 00133 Rome, Italy* 

<sup>3</sup>Yeastime Srl, Via Plutarco 48, 00012 Rome, Italy

<sup>4</sup>Department of Chemical Sciences and Technologies, University of Rome 'Tor Vergata', Via della Ricerca scientifica 00133 Rome, Italy

<sup>5</sup>Laboratory of Structural Biology, Department of Biology, University of Rome 'Tor Vergata', Via della Ricerca scientifica 00133 Rome, Italy

<sup>†</sup>deceased

\*fed.ort@icloud.com

Water is a potentially renewable resource, its long-term sustainability is threatened by the growing water footprint due to agricultural, industrial, and civil demand. Sustainable development policies are focused

at turning the current linear production system towards a circular one. In this scenario, wastewater represents a potential high valuable resource to upcycle. Microalgal-based platforms are promising biological alternatives to upcycle wastewater, but they still suffer from drawbacks related to the need to optimize biomass growth and target bio-compound yield.

This work investigates the potential to exploit mechanical stress for the optimization of biomass production and lipid yield for biofuel production from two green microalgae selected for their high resistance to stress and capability to grow on wastewater: *Desmodesmus* sp. and *Chlorella vulgaris*. To induce abiotic stress in a reliable way, we developed an *ad hoc* platform: photo-sonobioreactor. The latter allows, for the first time, the induction of mechanical stress for reproducible studies in microalgae intensive cultures.

Preliminary results showed that mechanical stress can influence not only biomass production but also lipid yield: indeed, high-power mechanical stress resulted in final biomass increase, meanwhile, low-power mechanical stress increased lipid yield. These data suggest a tradeoff of the effect that ultrasound induced mechanical stimulation triggers on biomass production and bio-compound level.

This study proposes mechanical stress as a compelling path that may unlock the potential of microalgae for wastewater upcycling.

# Multipurpose use of microalgae for sustainable agriculture as bioremediator of hydroponic greenhouse effluent and bioagrichemicals

Sirin, S.\*, Borhan, E., Salazar, J., Chovancek, E., Jokel, M., Allahverdiyeva, Y.

Molecular Plant Biology Unit, Department of Life Technologies, Faculty of Technology, University of Turku, Turku, Finland \*sesiri@utu.fi

Agricultural nutrient pollution has significant negative impacts on the environment, leading to eutrophication and ecosystem changes. To address this issue, we investigated the potential of microalgae, for bioremediation of hydroponic greenhouse effluents under Nordic environment. Moreover, we explored the reuse of microalgae as novel bio-based agrichemicals (e.g. biostimulants, biopesticides) to promote sustainable agriculture.

We screened several microalgae from the NordAqua Nordic Culture Collection Database and selected *Tetradesmus obliquus* for pilot scale cultivation. In controlled greenhouse trials conducted in a tubular photobioreactor during two seasons, we achieved 100% removal of nitrogen and phosphorus and satisfactory biomass accumulation.

We designed a phenotyping pipeline with two different assays to screen plant growth effects of several microalgal extracts. We observed an increase of 8-13% in *Arabidopsis* root growth and 12-15% in lettuce yield. Next, we integrated a stepwise extraction approach that resulted in  $\sim$ 30% increase in root growth of model plant, which is comparable to commercial plant stimulants. We also screened several strains from the Nordic database for their anti-fungal activity against several plant-pathogens (i.e., *F. oxysporum, P. cactorum*) and identified some potential candidates. The best extract was analyzed on strawberry leaves infected with *P. cactorum* and successfully slowed down the progression of the infection.

From a circular bioeconomy perspective and in response to the growing trend in sustainable agriculture, our approach advocates more efficient and sustainable closed-loop circular economy model, particularly for Nordic agriculture.

## Optimized extraction and separation processes for seaweed-derived polysaccharides and polyphenols for industrial applications

Tretiak, S.<sup>1\*</sup>, Hotchkiss, S.<sup>2</sup> & Popper, Z.A.<sup>1</sup>

<sup>1</sup>University of Galway, Ireland <sup>2</sup>CyberColloids, Carrigaline, Co. Cork, Ireland \*s.tretiak1@nuigalway.ie

The value of macroalgae as a source of biologically active compounds is becoming increasingly recognized throughout Europe and globally. Sustainable methods of extracting bioactive compounds are essential to supply the increasing demand for macroalgae as a functional material. Polysaccharides present in brown macroalgae are of increasing interest for biomedical- (e.g. laminarins and fucoidans) and food- (alginates) applications.

Pure forms of bioactive substances are highly valuable for commercial applications, thus optimising separation and purification methods are of industrial importance. Using sixteen different methods, we optimized the extraction of laminarin, fucoidan, and alginate from *Ascophyllum nodosum*. Methods included microwave-assisted extraction, autoclave-based hydrothermal-assisted extraction, extraction at different temperatures and with different solvents. We compared the yields obtained by these methods with those obtained by conventional methods. Extracts protein-, polysaccharide- and phenolic- content was assessed colorimetrically and the cost efficiency of each method was correlated with the yield efficiency to determine the most efficient methods for extracting polysaccharides from *Ascophyllum*.

Brown seaweeds contain a range of polyphenols including phlorotannins that are commonly coextracted with polysaccharides. The polyphenols impart astringency and bitter taste limiting food applications of the polysaccharides. Established methods for separating polysaccharides from polyphenols are costly and not food-friendly. We tested three techniques to reduce the level of phenolics in the extracts using nontoxic compounds; 1) polyvinylpolypyrrolidone, used to clarify beverages, 2) isinglass, used in the brewing industry, and 3) bovine serum albumin as an alternative to isinglass. However, none of the methods resulted in cost-efficient reduction of the phenolics:polysaccharide ratio of the extracts.

## Microalgae cultivation system based on additive manufacturing technology

Wishkerman, A.

Faculty of Marine Sciences, Ruppin Academic Center, Israel asherw@ruppin.ac.il

Microalgae are a source of plant-based products such as pigments, lipids, and carbohydrates. Although algae cultivation techniques have evolved and improved, much research is still needed to better understand these organisms and their biochemical and molecular mechanisms. Like other photosynthetic organisms' cultivation methods, there is a growing need for affordable, flexible, and easy to control research systems.

Additive manufacturing revolutionized design and development processes as it became widely available, allowing the creation of complex shapes, enabling mass customization, supporting low-volume manufacturing and distributed production. The emergence of opensource designs and electronics components combined with 3D printing applications have the potential to open new methods for plant cultivation.

The work describes a cell culture system that can be mounted on an orbital shaker or positioned on a shelf. It is based on low-cost commercial off-the-shelf components for low production cost, simplicity, design flexibility. The system utilizes programmable lightemitting diodes (LEDs) for application of different light patterns, intensity, and duration. The system is not limited to microalgae and can be used as an experimental platform for understanding the effect of light on other plant cells and other small sized organisms/microorganisms.

## Assessment of methane and hydrogen production from the brown seaweed Sargassum

Zhao, Y.1\*, Bourgougnon, N.2, Lanoisellé, J.-L.1 & Lendormi, T.1

<sup>1</sup>Univ. Bretagne Sud, UMR CNRS 6027, IRDL, F-56300 Pontivy, France <sup>2</sup>Laboratoire de Biotechnologie et Chimie Marines, Université Bretagne Sud, EMR CNRS 6076, IUEM, 56000 Vannes, France. \*yiru.zhao@univ-ubs.fr

For 10 years, the unusual massive inundations of brown seaweed *Sargassum* have been reported causing strong impact on the local economy, tourists, and the environment. Except utilization as compost in Guadeloupe and Martinique, this underexploited biomass has difficulty in finding ways of valorization. The objective of this study is to examine the BioMethanogenic Potential (BMP) and Bio-Hydrogen Potential (BHP) of the Caribbean *Sargassum* from fresh harvested, stranded, or composted biomass, along with setting-up of experiments in sequential continuous mode for a stable hydrogen production.

The BMP and the BHP of different algal biomass were determined using an Automatic Methane Potential Test System. Kinetic parameters were determined using the Gompertz model, Tukey's test was carried out to determine whether there was a statistical difference in fermentation performances. A significantly higher BMP value was obtained from harvested seaweed (136 NL CH<sub>4</sub>-kg VS<sup>-1</sup>) in comparison with other varieties, which remains high for data obtained from *Sargassum*. In terms of biodegradability, a descending order was observed (harvested-42%, stranded-26%, composted-11%). The BHP values, on the order of 10 NL H<sub>2</sub>-kg VS<sup>-1</sup>, appear low due to the complex structure of seaweed. Otherwise, a two-stage anaerobic digestion was observed in BHP test which highlights the importance of pH control.

This work allows a biodegradability assessment over time and a rough estimation of the energy production in a biorefinery scheme of seaweed. In the long term, we aim to propose a technically feasible alternative, for large-scale energy recovery of the co-products resulting from the biorefinery of Caribbean algae.

#### SYMPOSIUM 16 "Advances in algal production processes"

# Valorization of Spirulina's biomass as biostimulant in agriculture : development of a green product to improve sustainability and circular economy

Di Cave, A.<sup>1,2</sup>\*, Savio, S.<sup>1</sup>, Ortenzi, F.<sup>1,2</sup>, Rugnini, L.<sup>1</sup>, Migliore, G.<sup>3</sup>, Frassine, D.<sup>2,4</sup>, Scuderi, F.<sup>4</sup>, Braglia, R.<sup>4</sup>, Bruno, L.<sup>1†</sup> & Congestri, R.<sup>1</sup>

<sup>1</sup>Laboratory of Biology of Algae, Department of Biology, University of Rome Tor Vergata, Via della Ricerca scientifica 00133 Rome, Italy

<sup>2</sup>*PhD Program in Evolutionary Biology and Ecology, Department of Biology, University of Rome Tor Vergata, Via della Ricerca scientifica 00133 Rome, Italy* 

<sup>3</sup>ENEA-C.R. Casaccia, via Anguillarese, 301-- 00060 Rome, Italy

<sup>4</sup>Botanic Garden, Department Biology, University of Rome 'Tor Vergata' Via della Ricerca scientifica 00133 Rome, Italy

† deceased

\*alberta.dicave@gmail.com

This research aims to develop a plant biostimulant to support sustainable farming and contribute to the circular economy by utilizing residual algal biomass from Spirulina production process. Spirulina (*Arthospira platensis*) is already used as a food and feed integrator and is highly valuable for its phycocyanin content. Fertilization is indeed an important practice to improve crop yield but can be harmful for human, animal, and vegetable safety, and for the environment. In this scenario, plant biostimulants have demonstrated to enhance natural plant nutrition processes and reduce the need for nutrient demand and can be a sustainable alternative to chemical fertilizer.

We analyzed not only the residual algal biomass from phycocyanin extraction, but also pre- extraction one, to determine its quality as potential plant biostimulant. We assessed total nitrogen, phosphorus, and lipid content, and we found no significant differences in the chemical composition. To evaluate the action of the residual biomass, tests were conducted on edible plants (*Chycorium intybus*, L) with both residual, and pre-phycocyanin extraction biomass. The effectiveness was evaluated through plants morphometric measurements and by analyzing total pigment content. Preliminary results showed that the potential of the residual Spirulina biomass has no significative differences when compared to the pre-extraction one, and that the residual biomass can be used as a natural plant biostimulant. This study demonstrates the potential for Spirulina bioproduct upcycling to develop sustainable agricultural practices and contribute to reach a circular economy model. This research supports the "Biodiversity 2030" principles, and the "Farm to Fork" strategy, which aim to make agri-food systems fully sustainable, by proposing a natural alternative to conventional fertilizers that can optimize production and increase plant's tolerance to abiotic stress.

## Bubbling for light: fighting microalgae biofilms development in photobioreactors

Fanesi, A.\*, Martin, T. & Lopes, F.

Laboratoire Génie des Procédés et Matériaux (LGPM), CentraleSupélec, Université Paris-Saclay, 91190 Gif-sur-Yvette, France \*andrea.fanesi@centralesupelec.fr

Biofouling in photobioreactors (PBRs) negatively impact the cultivation process by decreasing light penetration and therefore biomass productivity. Biofouling results from the adhesion of single microalgal cells, and a later development of mature biofilms, on the surfaces of a reactor. The threedimensional structure (i.e. spatial organization of cells) of such complex microbial communities strongly affects their cohesion and response to mechanical stresses and therefore may impact their resistance to cleaning procedures. Despite the importance of understanding biofouling in PBRs, the 3D structure of microalgae biofilms has been seldom characterized. In this study, we aimed at understanding how microalgae (a diatom and a cyanobacterium) colonize the surfaces of PBRs by studying in the shortterm (few hours) their adhesion dynamics and in the long-term (weeks) their development as a function of different hydrodynamic conditions aimed at reducing biofouling. The experiments were conducted in a flat-panel reactor and several hydrodynamic conditions were generated by air bubbling. Cell adhesion was monitored by time-lapse microscopy and video analysis and biofilm evolution by confocal laser scanning microscopy (CLSM) and optical coherence tomography (OCT). The results show that the adhesion dynamics are species-specific and that shear-stress generated by bubbles helps creating lowbiofilm areas that improve light penetration. In these areas the biofilms appear to exhibit different 3D structures and a strong resilience to shear-stress is also identified. Overall, studying biofilm properties in PBRs allows a better comprehension of such complex microalgae lifestyle and helps improving bioprocesses stability and productivity.

## The Cellular mechanism of chlorosis in *Pyropia yezoensis* and the development of resistant strains

Kim, R.\*, Moon, J.S. & Kim, G.H.

Department of Biological Sciences, Kongju National University, Korea krw6531@kongju.ac.kr

*Pyropia* accounts for the largest share of the seaweed aquaculture industry and is an important aquatic product in Korea with an export volume of 30,000 tons and a value of \$700 million, as of 2022. However, production still does not meet demand for a variety of reasons. Among them is "chlorosis" caused by nutrient deficiency in seawater that causes enormous economic losses to *Pyropia* aquaculture. Our understanding of the cellular mechanisms that occur during chlorosis is limited. Recently, it has been reported that plants mediate reactiveoxygen-species (ROS) production, through intracellular NADPH-oxidase enzymes during abiotic stress, and that ROS is involved in chlorosis and apoptosis. We investigated the involvement of NADPH-oxidase generated ROS during the chlorotic process in *Pyropia yezoensis*. We localized ROS in thalli using DCFH-DA, and treated algae with the NADPHoxidase inhibitors ascorbic acid and DPI to determine their effects. Our results showed a large accumulation of ROS in thalli that increased over time during chlorosis progression. When thalli were treated with the NADPH-oxidase inhibitors, chlorosis was inhibited. Based on these results, selection of strains was undertaken by selecting varieties that showed lower ROS accumulation, resulting in lower effects of chlorosis. The development of chlorosisresistant strains is expected to greatly contribute to the improvement of *Pyropia* aquaculture in the future.

#### Bio-inspired materials for nutrient biocapture and antibiotics biodegradation in wastewater: microalgal cells immobilized on chitosan-based carriers

Vasilieva, S.G.<sup>1,2</sup>, Lukyanov, A.A.<sup>1\*</sup>, Lobakova, E.S.<sup>1</sup>, Grigoriev, T.E.<sup>3</sup>, Antipova, C.G.<sup>3</sup>, Chivkunova, O.B.<sup>1</sup>, Scherbakov, P.N.<sup>1</sup> & Solovchenko, A.E.<sup>1,2</sup>

<sup>1</sup>Faculty of Biology, Lomonosov Moscow State University, Russia

<sup>2</sup>Institute of Natural Sciences, Derzhavin Tambov State University, Russia

<sup>3</sup>Laboratory of polymeric materials, National Research Center "Kurchatov Institute", Russia

\*loockart@mail.ru

Immobilization of microalgal (MA) cells on various carriers is a widely accepted approach facilitating the biomass harvesting and protecting the cells from unfavorable environment. The immobilized MA often outperform their freely suspended counterparts in biomass accumulation, biodestruction of pollutants and nutrient removal.

The chlorophyte strain *Lobosphaera* sp. IPPAS C-2047 served as the object in the present work. We tested chitosan-based polycationic polymeric materials obtained by cryopolymerization of chitosan with glutaraldehyde which possess a high immobilization efficiency and supported the prolonged cultivation of immobilized MA without impairing their growth and photosynthetic activity. The nutrient removal capacity of the chitosanimmobilized *Lobosphaera* cells was significantly higher as compared to that of the suspended cells. The specific removal rates of inorganic phosphate and nitrate by the suspended microalgae cells comprised 0.6 and 3.2 mg mg<sup>-1</sup> Chl d<sup>-1</sup>, while the chitosan-immobilized *Lobosphaera* cells consumed phosphate and nitrate at rates 1.3 and 4.5 mg mg<sup>-1</sup> Chl d<sup>-1</sup>, respectively. The rate of ceftriaxone degradation by immobilized culture was 2.5-3.0 times higher as compared to suspended MA, which can be explained both by partial adsorption of ceftriaxone by chitosan and by greater efficiency of ceftriaxone biodegradation by immobilized MA.

During 14 d experiment chitosan-based carries demonstrated high mechanical and biological stability, but after 3 weeks of incubation the signs of polymer biodegradation were observed by electronic microscopy.

Collectively, the biodegradable and non-toxic cross-linked chitosan-based polymers were shown to be environment-friendly materials providing the enhancement of ceftriaxone degradation and nutrients bioremoval from wastewater by immobilized MA.

#### Chlorella vulgaris cultivation in low-cost photo-bioreactors

Mente, M.S.<sup>1\*</sup>, Papadimitriou, A.<sup>1</sup>, Lioga, A.<sup>1</sup>, Flora, E.<sup>1</sup>, Nerantzis, E.<sup>2</sup>, Tsirliganis, N.C.<sup>2</sup>, Kazakis, N.A.<sup>2</sup>, Mountourakis, F.<sup>3</sup>, Papazi, A.<sup>3</sup>, Stamatis, N.<sup>1</sup>, Kotzabasis, K.<sup>3</sup> & Orfanidis, S.<sup>1</sup>

<sup>1</sup>Fisheries Research Institute (ELGO-DIMITRA), 64007 Nea Peramos, Kavala, Greece

<sup>2</sup>Laboratory of Archaeometry and Physicochemical Measurements, Athena - Research and Innovation Center in Information, Communication and Knowledge Technologies, P.O. Box 159, Kimmeria University Campus, 67100 Xanthi, Greece

<sup>3</sup>Department of Biology, University of Crete, Voutes University Campus, GR-70013 Heraklion, Crete, Greece \*melpo.mente@gmail.com

This study investigated photosynthetic efficiency, biomass production, pigment, and lipid content of freshwater microalga *Chlorella vulgaris* Beijerinck 1890, cultivated in low-cost photobioreactors

placed in a greenhouse. The choice of semi-closed inclined cascade photobioreactor was made in order to achieve maximum absorption of photosynthetic irradiance, with minimal contamination, using innovative  $CO_2$  micro-bubble technology developed inhouse. Fully-closed inclined cascade photobioreactor with direct addition of 100%  $CO_2$  was also used.

The treatments included freshwater, semi-salty water, addition of industrial waste and different ways of CO<sub>2</sub> supply. Furthermore, due to the exposure of the photobioreactor to temperature and light changes, environmental factors such as pH, irradiance, and temperature had constantly been measured via data logger. Six experiments are shown and are being compared in this study. Maximum *C. vulgaris* production was 1.8 g/L, maximum Chlorophyla content 9.91 mg/ml and maximum lipid percentage 18% of dry biomass.

Based on the results of fatty acid analysis, the ratio of polyunsaturated and saturated fatty acids (PUFA/SFA >1) and the ratio of omega 6 and omega 3 polyunsaturated fatty acids ( $\omega 6/\omega$ -3 = 0.40 ± 0.12), as well as the Index of Atherogenicity (IA = 0.34 ± 0.07) and Index of Thrombogenicity (IT = 0.09 ± 0.01) were studied.

It was found that *C. vulgaris* had high nutritional value and benefits for human health. Alongside, it can be effectively cultivated in fully and semi-closed inclined low-cost photobioreactors outdoors, using freshwater and semi-salty water. Finally, *C. vulgaris* grew optimally in industrial waste so it can be used as biorefinery tool.

#### A microalgal consortium for the remediation of ammonium-rich wastewaters: species proportion variability and physiological analysis

Mollo, L.\*& Norici, A.

Laboratory of Algal and Plant Physiology, Polytechnic University of Marche, Italy \*lorenzo.mollo@pm.univpm.it

Ammonium rich wastewater, e.g. digestate, is an important source of nutrients such as nitrogen and phosphorous which could be used by algae in a wastewater treatment plant. While being a sustainable source of nutrients, nitrogen in the form of ammonium is usually toxic to most of the photosynthetic microorganisms since it affects the photosynthetic apparatus even at low concentrations.

A microalgae consortium composed of three green-algae (*Tetradesmus obliquus, Chlamydomonas reinhardtii, Auxenochlorella protothecoides*) was used to evaluate the effect of increasing NH<sub>3</sub>/NH<sub>4</sub><sup>+</sup> concentrations (1.5 to 50 mM of nitrogen). Growth performance, pigment and protein concentrations, C/N ratio,  $\delta^{13}$ C,  $\delta^{15}$ N and Dual Pulse Amplitude Modulation (PAM) analysis were carried out to obtain a complete and critical view of the consortium response. Moreover, a cytofluorimeter analysis provided further details on consortium composition.

While growth rate was generally higher for algal cultures fed with  $NH_3/NH_4^+$  if compared with the control-ones (in  $NO_3^-$ ), algal density was lower even at the lowest concentration tested. Similarly, chlorophyll concentrations were far lower than the control ones proving a toxicity to the photosystems, validated also by PAM analysis.

Proportion among the species in term of cell density shifted at the expense of *T. obliquus* with increasing nitrogen concentration. The shift was even more pronounced when  $NH_3/NH_4^+$  was the N-chemical form. Results open to new perspectives in the field of wastewater remediation giving new insights on the consortium resilience in response to ammonium toxicity and they allow scientists to better monitor algal consortia and modulate growth parameters during this application.

#### A consistent and reliable system for studying eucheumatoid biology

Narayanan, R.A.<sup>1\*</sup>, Karthick, P.<sup>1</sup>, Shashibhushan, N.B.<sup>1</sup>, Kambey, C.S.B.<sup>2</sup> & Critchley, A.<sup>3</sup>

<sup>1</sup>Sea6 Energy Pvt Ltd., 1<sup>st</sup> Floor, C-CAMP, NCBS-TIFR, GKVK Post, Bellary Road, Bangalore 560065, India. <sup>2</sup>PT Sea6 Energy Indonesia, Nusa Dua, Bali, Indonesia.

<sup>3</sup>Verschuren Centre for Sustainability in Energy and Environment, Sydney, Cape Breton, Nova Scotia, Canada. \*narayanan@sea6energy.com

Tropical eucheumatoid cultivation constitutes a major portion of world macroalgal production for hydrocolloids and other applications. Over the recent past, significant reduction in productivity has been observed in many tropical growing areas including Philippines, Indonesia and Tanzania. This has been attributed to various factors including loss of growth vigour due to repeated clonal propagation, lack of genetic diversity, disease and global climate change. To understand and improve eucheumatoid cultivation, it is imperative to have small-scale, laboratory systems for studying physiology under controlled conditions. Such an assay system will help understand nutritional needs, develop disease models and investigate solutions in a fast and easy manner. *Kappaphycus alvarezii* was cultured in 2L glass beakers in natural seawater under gentle aeration and 12:12 D/N cycle. The thallus was tied to a string and suspended from the top. Consistent daily growth rates of more than 2% was observed over a period of 30 days and the thallus could be maintained for up to 80 days. Supplementation of seawater with nitrogen (Ammonium or Nitrate) enhanced growth (upto 4.5% DGR), while addition of phosphate inhibited growth. This system can be used to identify essential nutrients, nutritional factors involved in growth and disease and measure uptake kinetics of eucheumatoid seaweeds at low cost under simple operating conditions in a repeatable manner.

## Reduced night biomass loss in large-scale mixotrophic algal cultivation

Nham, Q.\* & Lindehoff, E.

*Linnæus University, EEMiS (Ecology and Evolution in Microbial model systems), Sweden* \* quyen.nham@lnu.se

Night biomass loss in algal cultivation, due to respiration and cell mortality, can reduce 53% of the biomass synthesized in daylight. The negative effects can be counteracted by adding organic carbon (C), e.g. mixotrophic cultivation, to supply C and energy for cell maintenance and division during darkness. The potential of mixotrophic cultivation to reduce biomass loss remains untested in outdoor large-scale conditions using raw industrial waste streams. We investigated night biomass loss in cultivations of the alga *Monoraphidium minutum* KAC90 in outdoor 1 m<sup>3</sup> raceway ponds, using flue gas condensate (nitrogen source) and cheese whey (phosphorus (P) and organic C source) for the mixotrophic treatment or sodium monophosphate (P source) for the autotrophic control. The experiment was performed in the autumn (October-November 2022) at temperatures of 7.8-15.8°C, maximum daylight intensities of 46-586 µmol m<sup>-2</sup> s<sup>-1</sup>, and daylengths of 7-9 hours. During six cultivation cycles, biomass was sampled for dry weight, respiration measurement, biochemical composition and identification of microalgae and bacteria (18S rDNA, 16S rDNA). Results so far indicate that the autotrophic control (8-25 mg/L COD) had a night biomass loss of 5%±15%, while the mixotrophic treatment had a night biomass gain of 33%±16% in the presence of high initial organic C (239-613 mg/L COD). However, at low organic C (36-189 mg/L COD), the mixotrophic treatment had a night biomass loss of 10%±9%, comparable to

that of the autotrophic control (Wilcoxon test, p>0.05). Thus, night biomass loss in algal cultivations was prevented when organic C was sufficient (COD $\geq$ 239 mg/L).

## SYMPOSIUM 17 "Public-Private partnerships in algal research"

## Stimulating algae cultivation in Spain: tools developed by the INNOVALGA project

Malta, E.-j.<sup>1\*</sup>, Macías, M.<sup>1</sup>, Gómez-Pinchetti, J.L.<sup>2</sup>, Hernández, I.<sup>3</sup>, Iglesias, P.<sup>4</sup>, Bermejo, R.<sup>5</sup> & Agraso-Martínez, M.M.<sup>1</sup>

<sup>1</sup>CTAQUA- Fundación Centro Tecnológico Acuicultura de Andalucía, Spain
 <sup>2</sup>Banco Español de Algas, Fundación Canaria Parque Científico Tecnológico de la Universidad de Las Palmas de Gran Canaria, Spain
 <sup>3</sup>Department of Biology, Faculty or Marine and Environmental Research, University of Cadiz, Spain
 <sup>4</sup>ANFACO-CECOPESCA, Vigo, Spain
 <sup>5</sup>Department of Ecology, Faculty of Sciences, Malaga University, Spain
 \*e.malta@ctaqua.es

The European algae sector has a large growth potential and it is acknowledged as an important sector of the EU blue bioeconomy. Last year, the European Commission published an action plan with the aim to stimulate algal biomass production in the EU ("Towards a Strong and Sustainable EU Algae Sector", COM 592-2022).

Certain areas of Spain have a long tradition of seaweed biomass collection and procession, whereas algae cultivation is of a more recent date. However, various barriers for algae cultivation have been recognized (technical, economic and administrative) that are inhibiting the development of an algae sector in Spain. The INNOVALGA project, launched in January 2022, plans to contribute to the growth of the blue economy by boosting an emerging and sustainable industry for the cultivation and use of macroalgae and microalgae.

Here we present a toolbox for both present and future algae farmers developed in INNOVALGA. This toolbox consists amongst others of a catalogue, identifying the micro and macroalgae species with potential use and commercial cultivation in Spain based on a critical analysis of species and cultivation methods. Furthermore, an assessment of recent rules and regulations regarding the production and processing of algae (macro- and microalgae), both at regional, national and European level has been carried out and presented in a practical guide for farmers. Based on these, a roadmap with recommendations for both the sector and for autonomous and national administrations to promote the development of the algae sector has been established.

### SYMPOSIUM 18 "Algae and the general public" + SYMPOSIUM 19 "Algae, education and career pathways"

## Studying marine macrophytes through citizen science. The case of the Big Seaweed Search Project-Mexico

Galindo-De Santiago, M.C.<sup>1,2</sup>, Brodie, J.<sup>3\*</sup>, Vázquez Delfín, E.<sup>1,4</sup>, ParedesChi, A.<sup>2,6</sup>, Ríos Vázquez, A.<sup>1</sup> & Benavides Lahnstein, A.I.<sup>5</sup>

<sup>1</sup>Escuela Nacional de Estudios Superiores, Unidad Mérida, Universidad Nacional Autónoma de México, Yucatán, México.

<sup>2</sup>Facultad de Ciencias, UMDI Sisal, Universidad Nacional Autónoma de México, Yucatán, México. <sup>3</sup>Natural History Museum, Science, London, UK.

<sup>4</sup>Laboratorio de Ficología Aplicada, Departamento de Recursos del Mar, CINVESTAV Mérida, Yucatán, México.

<sup>5</sup>Angela Marmont Centre for UK Biodiversity, Natural History Museum, London, UK.

<sup>6</sup>CONACYT, Facultad de Ciencias, UMDI Sisal, Universidad Nacional Autónoma de México, Yucatán, México.

\*j.brodie@nhm.ac.uk

The study of marine macrophytes that arrive on beaches has gained importance in the last decade due to massive inundations of pelagic Sargassum ('Golden Tides'), events that have caused serious environmental and socio-economic problems in the Mexican Caribbean. Through Citizen Science, we record the variation in species composition of the beach-cast biomass and the relative abundance of the species by encouraging collaboration of local participants from a community of the Mexican Caribbean (Puerto Morelos) with scientific researchers. This project was inspired by the Big Seaweed Search (BSS), where citizens record 14 macroalgae around UK coasts, Species were chosen as indicators of environmental change, including rising sea surface temperatures, ocean acidification, and the spread of non-native species. Big Seaweed Search Mexico (BSSMx) was adapted to local socio-cultural and environmental conditions, focusing on beach-cast species. From April 2022 to January 2023 during three climatic seasons (dry, rains and 'nortes'), 42 macroalgal species and three seagrass species were recorded. Three species of pelagic Sargassum (S. fluitans, S. natans I and S. natans VIII) and one seagrass (Syringodium filiforme) dominated the strandings. With the information obtained, a baseline can be established to propose long-term monitoring programs, fostering local educational activities and expansion of data collection through Citizen Science. Additionally, a change was observed in participant's perception of the macroalgal strandings, highlighting that it is possible to promote educational opportunities in relation to the understanding of Sargassum events and promote community actions with a climate change mitigation perspective.

#### Colorimetric analysis of algal pigments from the Alboran Sea

Yebra, A.<sup>1\*</sup>, Cuberos, L.M.<sup>2</sup>, Contreras, Y.R.<sup>2</sup>, Ballesteros, J.C.<sup>2</sup>, Bailón, R.<sup>3</sup>, Pula, H.J.<sup>4</sup>, De La Rosa, J.C.<sup>5</sup> & Bolívar, F.C.<sup>2</sup>

<sup>1</sup>Optics Laboratory. Faculty of Science, Granada <sup>2</sup>Ligalism Laboratory. Faculty of Fine Arts, Granada <sup>3</sup>Chemical engineering Laboraty. Faculty of Science, Granada. <sup>4</sup>Zoology Laboratory. Faculty of Science, Granada. <sup>5</sup>*Phycology Laboratory. Faculty of Science, Granada.* \*ayebra@ugr.es

The Alboran Sea, due to its rich algal and cultural biodiversity, is an excellent place to study from a scientific and artistic point of view. Furthermore, algae are a source of raw material for a multitude of applications, due to the great diversity of chemical components that can be obtained from them. Of greatest interest are lipids, polyunsaturated fatty acids, cellulose, pigments and dyes.

In artistic techniques and conservation-restoration applications have hardly been developed. For this reason, our research group HUM 1031: Ligalism (Arts-Sciences), with extensive experience in the artistic use of algae from the Alhambra, is carrying out analyses with metabolites extracted from algae native to the coasts of the Alboran Sea, with the aim of creating new artistic and conservation-restoration materials, mainly pigments and dyes of natural origin, sustainable, non-toxic and with a high cultural importance for the protection and conservation of our coasts.

To carry out this study, two strains of native green, red and brown algae from the coast of Granada have been selected, and their algal pigments have been extracted and their metabolites stabilised. Likewise, colour control analyses are being carried out on these algal pigments after undergoing a process of accelerated ageing with different degradation factors, and studying the differences in colour with the CIELab and CIEDE2000 formulas, with the final objective of obtaining natural and biosustainable products for artistic use and conservation-restoration.

#### PHYCO-ART. In search of the lost technique

Cuberos, L.M.<sup>1\*</sup>, Yebra, A.<sup>2</sup>, Contreras, Y.R.<sup>1</sup>, Bolívar, M.<sup>1</sup>, Romero, J.<sup>1</sup>, Bailón, R.<sup>3</sup> & Bolívar, F.C.<sup>1</sup>

<sup>1</sup>Ligalismo Laboratoy. Painting Dept. University of Granada. Avda. Andalucía s/n, 18071, Spain.
 <sup>2</sup>Optics Laboratory. Optics Dept. University of Granada. Avda. Fuente nueva s/n, 18071, Spain.
 <sup>3</sup>Techné Laboratory. Chemical Engineering Dept. UGR Avda. Fuente nueva s/n, 18071, Spain.
 \*lorenamaria@ugr.es

In the patrimony, tourist and artistic sectors, colour is a fundamental element, since the visual arts cannot do without the chromatic spectrum and its modes of representation. Our research group HUM-1031 LIGALISMO (Arts & Sciences), through the project "New colourings for the ecological and digital transition" and financed by the State Plan for Scientific and Technical Research and Innovation, is studying the metabolites extracted from the algae belonging to the monumental complex of the Alhambra and the Generalife and which are treated as agents of biodeterioration, due to the high presence of water and high humidity in this environment, with the aim of creating new artistic materials, mainly pigments of natural origin, non-toxic, sustainable and important for the environment, a field that until now has been little studied.

Green algae, cyanobacteria and diatoms were selected from this monumental complex, as these microorganisms are capable of producing various colouring substances in yellow, green, orange and brownish-brown tones. Biomass has been created with them to subsequently extract their algal pigments and stabilise their metabolites. Currently, colourimetric control analyses are being carried out on these algal pigments after accelerated ageing with different degradation factors, such as temperature, light and humidity (studying colour differences with the CIELab and CIEDE2000 formulas), in order to know the resistance of these organic pigments, with the final objective of obtaining biosustainable and stable products for artistic use.

## PlanktoQuest: a virtual reality animation to explore inside phytoplankton

#### Decelle, J.

*Cell and Plant Physiology laboratory, CNRS, Grenoble (France)* johan.decelle@univ-grenoble-alpes.fr

Virtual reality is a very efficient technology to communicate with the general public and has the power to engage and inspire students in education. We recently developed a new virtual reality animation to enter and explore inside marine microalgae (phytoplankton). We used 3D models from real 3D electron microscopy data from different organelles of microalgae (chloroplast, mitochondrion, nucleus) covering different groups, such as coccolithophores, dinoflagellates, haptophytes. Users are active in this animation by just using their hands (no controllers): they can choose and catch a swimming plankton, dissect the organelles, feed the plankton, and do a 360° voyage inside a cell.

PlanktoQuest will be presented at the conference and attendees will be able to try the immersive animation with the headset.

Teaser: <u>https://www.youtube.com/watch?v=-ydI1CAO6GU</u>

## Identification of phytoplankton using artificial intelligence: a case study on freshwater bioindicators

Hodač, L.<sup>1</sup>, Carreño, E.<sup>2</sup>, Dunker, S.<sup>3,4</sup>, Mäder, P.<sup>2,4,5</sup>, Jamroszczyk, M.<sup>6</sup>, Lorenz, M.<sup>6</sup>\*, Meier, S.<sup>7</sup>, Dürselen, C.-D.<sup>7</sup>, Šubrt, D.<sup>8</sup> & Wäldchen, J.<sup>1,4</sup>

<sup>1</sup>Max Planck Institute for Biogeochemistry, Department of Biochemical Integration, Germany

<sup>2</sup>Technical University Ilmenau, Data-intensive Systems and Visualization Group, Germany

<sup>3</sup>*Helmholtz-Centre for Environmental Research – UFZ, Department Physiological Diversity, Germany* 

<sup>4</sup>German Centre for Integrative Biodiversity Research – iDiv (Halle-Jena-Leipzig), Germany

<sup>5</sup>*Friedrich Schiller University Jena, Faculty of Biological Sciences, Germany* 

<sup>6</sup>University of Göttingen, Experimental Phycology and SAG Culture Collection of Algae, Germany <sup>7</sup>AquaEcology GmbH & Co. KG, Oldenburg, Germany

<sup>8</sup>Faculty of Science, Jan Evangelista Purkyně University in Ústí nad Labem, Czech Republic \*mlorenz@gwdg.de

The identification of phytoplankton species in inland waters is currently not easily accomplished by automated methods. While machine learning approaches have been explored, they have not yet been widely adopted. We aim to change this by bridging computer science and phycology, using recent advances in machine learning to automatically identify phytoplankton species with unique morphology, bioindication under the Water Framework Directive (WFD), and sound taxonomy. Focusing on the most common phytoplankton taxa listed as bioindicators in national taxa lists, we collect micrographs and associated DNA sequences (barcodes) from public databases such as GBIF, GenBank, and algal culture collections. This will provide insight into the morphological and genetic variability of bioindicator phytoplankton taxa. To automatically identify selected species from standard microscopy images, we perform deep learning experiments on thousands of microphotographs sampled from over 90 freshwater bodies in Europe, taking into account the variability of species in monoclonal cultures. As a first step, we are developing an informatics pipeline for automatic classification and detection of freshwater

chlorophytes, which can be extended to other algal groups. This technology can facilitate automated qualitative and quantitative analyses useful for biodiversity monitoring and citizen science projects.

#### Littoral monitoring for an experience of teaching and research

Pena-Martín, C.<sup>1\*</sup>, Terradas, M.<sup>2</sup>, Abellán, E.<sup>2</sup>, Fernández-Torquemada, Y.<sup>2</sup>, Mas, J.A.<sup>3</sup>, Navarro, N.<sup>3</sup>, Navarro-Martínez, P.C.<sup>4</sup>, Núñez, T.<sup>3</sup>, Orenes-Salazar, V.<sup>4</sup>, Scott, R.<sup>5</sup>, Del-Pilar-Ruso<sup>2</sup>, Y. & Valverde, M.<sup>2</sup>

<sup>1</sup>Dpto. Ciencias Ambientales y Recursos Naturales, University of Alicante, Spain
<sup>2</sup>Dpto. Ciencias del Mar y Biología aplicada, University of Alicante, Spain
<sup>3</sup>Dpto. Biología y Geología, Física y Química Inorgánica. U. Rey Juan Carlos, Spain
<sup>4</sup>Dpto. Ecología e Hidrología, Universidad de Murcia, Spain
<sup>5</sup>Fac. Educación, University of Alicante, Spain
\*carolina.pena@ua.es

Samplings that cover an extensive spatial and temporal scale are required in order to know the different levels of variability of biological communities, and their long-term trend, especially within the current context of global change. On this line, several volunteer initiatives have already been carried out to cover this temporal dimension (Bishop, Sherkin Island Marine Station Publications, 2003; Osborn et al., American Society of Civil Engineers, Reston, VA, 2005). This kind of experience implies, together with scientific data, a collective learning that contributes to the training of students and scientific dissemination and environmental awareness.

Our work started in September 2021, and are implicated three Universities (Alicante, Murcia and Rey Juan Carlos), from where research teachers and students are involved. Before starting the monitoring, training workshops were held for the students. The 5 studied localities are distributed along the Spanish Mediterranean coast, following a discontinuous cartography by transects, with a periodicity of 3 months, according to Dethier *et al.* (Mar. Ecol. Prog. Ser. 96: 93-100, 1993). Macrophytobenthos, phytoplankton and abiotic data are considered, and included in a common archive (Google Drive).

This project is open to more participants, expanding the geographical area studied. The experience, not only provide invaluable information for research (knowledge of community dynamics, detection and monitoring of vulnerable, bioindicator or invasive species), but also proves a tool for teaching and training students (taxonomy, sample designs, work in the field, data analysis), and social and scientific dissemination (importance and fragility of marine ecosystems, awareness, scientific papers, congress communications).

## Publications & Public Actions: How can Scientists respond to the Climate and Ecological Emergency?

Perrin, A.J.<sup>1\*</sup>, Thierry, A.<sup>2</sup> & Gardner, C.J.<sup>3</sup>

<sup>1</sup>University of York, United Kingdom <sup>2</sup>Cardiff University, United Kingdom <sup>3</sup>University of Kent, United Kingdom \*abi.perrin@york.ac.uk

Planetary heating and the destruction of ecosystems has precipitated a Climate and Ecological Emergency requiring an urgent response. The collective actions taken - or not taken - now will determine the conditions for life for every individual and every species on Earth. Our oceans and all other aquatic

ecosystems - the natural habitats for the algae we work on - are existentially threatened, as are the societal structures required for us to continue our scientific research. Faced with these realities we can feel powerless, but scientists are in a prime position to drive positive change amidst these deepening crises. This poster highlights the factors that give scientists particular importance, influence and investment and also identifies six broad types of action we can take. It aims to stimulate discussions about the role of science and academia in a rapidly changing world and how we can use our skills most effectively to facilitate and accelerate essential global, systemic and societal transitions.

# Twenty-Year Study of Algal Taxonomic Diversity in the Intertidal Zone of Hœdic Island: A Pedagogical Program for Life Sciences Students

Ten-Hage, L.<sup>1\*</sup>, Maumont, S.<sup>2</sup> & Roux, C.<sup>3</sup>

<sup>1</sup>Laboratoire d'Ecologie Fonctionnelle et Environnement, UT3/CNRS, France <sup>2</sup>Laboratoire Ecologie et Diversité Biologique, UT3/CNRS, France <sup>3</sup>Laboratore de Recherches en Sciences Végétales, UT3/CNRS, France \*loic.tenhage@univ-tlse3.fr

For the past 20 years, a pedagogical program has been conducted to study the taxonomic diversity of algae in the intertidal zone of Hœdic Island, Morbihan, France. The program, part of the Life Sciences Bachelor's degree at the University of Toulouse 3, is designed to provide students with practical experience of biodiversity and algae ecology in a natural environment. Students are introduced to the collection, classification, and identification of algae, as well as the evaluation of their ecological role in the marine ecosystem. As a result, each year, students identify nearly 80 species of algae collected in calm or rough environments. The data collected over the years have allowed for the tracking of annual variations in algae diversity. The program also aims to raise student awareness about the importance of marine biodiversity conservation and sustainable natural resource management. The results of this pedagogical program contribute to the understanding of marine biodiversity and algae ecology on Hœdic Island (a Natura 2000 site).

## When society meets science - results of the survey on cyanobacterial blooms

Wilk-Woźniak, E.<sup>1\*</sup>, Valskys, V.<sup>2</sup>, Messyasz, B.<sup>3</sup>, Gulbinas, Z.<sup>4</sup>, Krztoń, W.<sup>1</sup>, Walusiak, E.<sup>1</sup>, Łaciak, M.<sup>1</sup>, Budziak, M.<sup>1</sup>, Leska, B.<sup>5</sup>, Pankiewicz, R.<sup>5</sup>, Karosiene, J.<sup>6</sup>, Kasperoviciene, J.<sup>6</sup> & Koreiviene, J.<sup>6</sup>

<sup>1</sup>Institute of Nature Conservation Polish Academy of Sciences, al. Adama Mickiewicza 33, 31-120 Kraków, Poland,

<sup>2</sup>Institute of Biosciences, Life Sciences Centre, Vilnius University, Vilnius, Lithuania

<sup>3</sup>Faculty of Biology Adam Mickiewicz University, Poznań, Poland

<sup>4</sup>Nature Heritage Fund, Vilnius, Lithuania

<sup>5</sup>Faculty of Chemistry Adam Mickiewicz University, Poznań, Poland

<sup>6</sup>The Nature Research Centre, Vilnius, Lithuania

\*wilk@iop.krakow.pl

Citizen science could be a helpful tool that combines ecological research with environmental education. In community-based monitoring, data collection methods need to be improved to obtain more

representative results. The questionnaire proposed by the project "AlgaeService for LIFE" will facilitate understanding the knowledge gaps in society about cyanobacterial blooms, the threats posed by cyanotoxins, people's attitudes toward the problem, and the best sources for information dissemination in different communities. Most of the 1028 responses came from citizens in Poland and Lithuania, but the questionnaire also reached people in 14 other countries in Europe and Asia. Cyanobacterial blooms and toxic compounds of cyanobacteria were known to more than half of the respondents. They recognised negative effects on livestock, pets, domestic birds, poultry and fish. People get their knowledge mainly from TV, the radio, newspapers or professional seminars, but the other dissemination channels (social networks, news portals, etc.) also contribute to the rising awareness of certain groups of society. Understanding what information society needs and what channels can be used to reach them will help increase society's engagement and ensure data quality for another tool created as part of the project - the ArcGIS application interactive map.

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### SYMPOSIUM 20 "Coastal and freshwater systems under human pressure: status assessment, management and conservation"

#### The Moroccan Mediterranean Sea medelled by Ecopath model

Aboussalam, S.<sup>1\*</sup>, Khalil, K.<sup>2</sup> & Elkalay, K.<sup>2</sup>

<sup>1</sup>Laboratory of Applied Sciences for the Environment and Sustainable Development, Morocco <sup>2</sup>Laboratory of Applied Sciences for the Environment and Sustainable Development, Morocco salmaaboussalam@gmail.com

An Ecopath mass balance model was applied in the Moroccan Mediterranean Sea ecosystem to describe the structure, functioning and state of the system. In this work to explore the trophic interaction we consider 31 functional groups, containing 21 fish, 7 invertebrates, 2 primary producers and one dead group (detritus). The average trophic transfer efficiency for the entire system was 23%. Major standard indicators (PPT/TR=1.39, PPT/TB=14.83, TB/TST=0.02) suggest that the Moroccan Mediterranean Sea is a developing ecosystem. The structure of our system is based on high flows of respiration and consumption. The low values of FCI (6.91), SOI (0.19) and FML (2.80) indicate that our system is disturbed and have a more linear than web-like trophic structure. The Keystone Index and Mixed Trophic Impact analysis indicated that other invertebrate demersals, zooplankton and cephalopods showed a huge impact on other groups and they are recognized as a keystone species.

#### Development of disinfection protocols for fishing gear to minimize the impact of invasive seaweed *Rugulopteryx okamurae* (Dictyotales, Ochrophyta)

Altamirano, M.<sup>1\*</sup>, Núñez, Á.<sup>1</sup>, Loring, J.<sup>1</sup>, Sydorenko, A.<sup>1</sup>, Zanolla, M.<sup>1</sup>, De la Rosa, J.<sup>2</sup>, Rosas-Guerrero, J.<sup>1</sup> & Carmona, R.<sup>3</sup>

<sup>1</sup>Departamento de Botánica y Fisiología Vegetal, Facultad de Ciencias, Universidad de Málaga, Málaga, Spain

<sup>2</sup>Departamento de Botánica, Facultad de Ciencias, Universidad de Granada, Granada, Spain <sup>3</sup>Departamento de Ecología y Geología, Facultad de Ciencias, Universidad de Málaga, Málaga, Spain \*altamirano@uma.es

Since its identification in the Strait of Gibraltar in 2016, the asiatic brown seaweed Rugulopteryx okamurae has produced unprecedented environmental impacts. But what has caught the attention of public administrations and the media has been the millionaire economic impacts it produces on the fishing sector and for beach management. The Spanish national strategy for the control of the species has identified the fishing sector not only as the main sector affected by the species, but also as a key player in controlling its dispersal. Huge amounts of *R. okamurae* biomass are trapped in fishing gear, which facilitates its dispersal if it is not cleaned and disinfected before being used in other areas. For this reason, the complicity of the fishing sector in the fight against the species is urgent, as well as the development of protocols for disinfecting fishing gear to minimize the potential of fishing activities to disperse the species accidentally. This paper will show on the one hand the collaborations carried out with the fishing sector to work together in the management of the species, and on the other hand the results of different laboratory experiments for the development of fishing gear disinfection protocols. These experiments have been carried out by studying the effects on R. okamurae photosynthesis, estimated as fluorescence measurements, of different treatments combining different chemical products, at different concentrations and application time, as well as physical stress factors such as osmotic shocks and high irradiance. All this to identify treatments that guarantee a mortality of the species above 90% in the shortest possible time, with the lowest economic cost and as innocuous as possible for the user.

### Seaweeds in ports along the Channel-Atlantic Coast of France: factors influencing species assemblages and distribution

Burel, T., Anquet, A., Helias, M., Bizien, A., Le Duff, M. & le Garrec, V.

Univ Brest, CNRS, IRD, Ifremer, LEMAR, 29280 Plouzané, Brittany, France

Communities colonizing hard substrata in ports are of significant interest, as pontoons represent an artificial habitat that is not found in natural ecosystems. Pontoons provide a privileged environment for the colonization of non-indigenous species (NIS), which can be transported and dispersed through maritime traffic. This study aims at investigating the distribution of NIS within ports located along the Channel-Atlantic coast of France and understanding the factors influencing the formation of species assemblages on pontoons. A total of 15 ports were monitored using a new sampling protocol combining rapid assessment surveys in the field and microscopical observations.

The macroalgal communities found in ports were around 150 species. A larger part of the diversity was due to NIS in 13 %, which is higher than found in nearby marine habitats (7 % for maerl beds and seagrass meadows). We hypothesize that communities (1) show large discrepancies between ports, due to latitudinal factors with the occurrence of kelp species in the northernmost ports, and filamentous red in the southernmost ones, (2) experience smallscale variations, due to orientation, inner port/seaward gradient, and type of substratum, which affect their composition, (3) are influenced by anthropogenic factors such as the influx of maritime traffic contributing to higher concentration of NIS near introduction hotspots, such as the Bay of Arcachon and the Gulf of Morbihan.

Although the protocol seems effective in detecting NIS and has the potential to be used for monitoring as part of the Marine Strategy Framework Directive, calibration is required with neighboring regions to ensure its efficiency.

## Alien Invasions: Quantifying the invasiveness risk of macroalgae in the Azores

Gabriel, D.\*<sup>1</sup>, Martins, B.<sup>1</sup>, Ribeiro, C.<sup>1</sup>, Lorenzo Vilizzi, L.<sup>2</sup> & Fredericq, S.<sup>3</sup>

<sup>1</sup>CIBIO, University of the Azores, Portugal

<sup>2</sup>Department of Ecology and Vertebrate Zoology, University of Lodz, Poland

<sup>3</sup>Department of Biology, University of Louisiana at Lafayette, USA

\*daniela.l.gabriel@uac.pt

The presence of aliens or non-indigenous species (NIS) is a known threat to biodiversity, health, and economy, so it is of high importance that their invasiveness risk be quantified. The mid-northern Atlantic archipelago of the Azores encompasses 30% of the European Union exclusive economic zone and is known to host a high proportion of marine NIS.

An updated list of marine macroalgae classified as NIS in the Azores was produced, with 42 taxa (8.05%) presently recognized as non-native, whereas the more isolated islands in the Eastern Group presented lower numbers of NIS (4.25% and 6.25%). The two islands with the most used marina for transatlantic recreational sailing presented higher numbers of NIS (12.90% and 16.87%).

The Aquatic Species Invasiveness Screening Kit (AS-ISK), a decision-support tool consisting of 49 questions for the Basic Risk Assessment (BRA) and six for the Climate Change Assessment (CCA), was applied to the updated list above. The results indicate that 21 species pose a medium risk of invasion and 15 present a high risk under current climate conditions (BRA scores only). Based on the BRA+CCA scores, 20 species pose a medium risk of invasion and 16 a high risk. The Rhodophyceae presented the higher number of species classified as high risk, and the Chlorophyceae presented a higher percentage of species ranked as high risk.

Some considerations are provided for the management of species in their initial stages of establishment.

## Effect of mobile bottom trawling on microphytobenthic communities in the German Baltic Sea

Janßen, M.\* & Karsten, U.

University of Rostock, Applied Ecology and Phycology, Rostock, Germany \*marjan.janssen@uni-rostock.de

Mobile bottom fishing has a direct and indirect effect on benthic communities, sediment characteristics and nutrient fluxes at the sediment-water interface due to heavy fishing gear. Marine protected areas (MPA) are currently not exempt from bottom trawling and thus there are hardly any undisturbed areas in the Baltic Sea and almost no research to estimate the impact of bottom trawling on benthic marine habitats. The present study is part of a comprehensive joint project, which deals with the effect of bottom trawling on benthic and demersal ecosystem components, with our focus on microphytobenthic communities. Microphytobenthos, mostly dominated by diatoms, are primary producers and thus key players in the benthic community of marine soft bottoms. In the past years we collected sediment cores from several areas in the Baltic Sea (MPA and Reference) and examined microphytobenthic primary production and diversity (top 1-2 cm). For a quantitative and qualitative data analysis, we measured primary production, chlorophyll a content as well as sediment characteristics. With the development of new methods, we were for the first time able to measure the cores on the ship directly. The diatom communities were investigated morphologically via light microscopy and molecular markers using

high-throughput sequencing. We found benthic diatom species in all samples even as deep as  $\sim$ 30 m. The presented data show no significant differences for primary production, chl a, carbon and nitrogen content between reference and MPA indicating that bottom trawling should be excluded from all protected areas.

#### Phytoplankton and Ecological status of natural lakes in Greece

Nikolopoulou, I.<sup>1\*</sup>, Navrozidou, V.<sup>1</sup>, Moschandreou, K.<sup>2</sup>, Kemitzoglou, D<sup>1</sup> & Tsiaoussi, V.<sup>1</sup>

<sup>1</sup>Greek Biotope/Wetland Centre, The Goulandris Natural History Museum, Thessaloniki, Greece <sup>2</sup>Ministry of Rural Development & Food, Directorate General of Fisheries, Directorate of Aquaculture, Athens, Greece \*inikolop@ekby.gr

In accordance with the Water Framework Directive (WFD), Greece applies the Hellenic Phytoplankton Assessment System (HeLPhy) to classify the ecological status of natural deep warm monomictic and shallow polymictic lakes. HeLPhy responds to eutrophication pressure. The objectives of the present study were a) to present the inter-annual variations of phytoplankton composition and biovolume and of the HeLPhy values in 14 natural lakes from 2016 to 2021 and b) to investigate similarities of lakes based on phytoplankton taxonomic groups. Biovolume values of the main phytoplankton taxonomic groups and HeLPhy values (mean±SD) for 2016-2021 were presented for each lake. The 14 lakes were classified in high, good, moderate and poor status. Seven lakes met the WFD good status target according to HeLPhy. Deep lakes showed higher ecological status than shallow ones. Non-Metric Multidimensional Scaling (NMDS) and one-way analysis of similarity percentages were applied to visualize similarities and to identify the taxonomic groups responsible for the discrimination in lakes. Four groups of lakes were discerned in the NMDS plot (stress value: 0.05). Lakes spread out in the ordination plot, following mostly the ecological gradient of HeLPhy. The pattern of their distribution also seemed to follow the depth gradient. Cyanobacteria and Bacillariophyceae contributed most to the average dissimilarity of lakes. In order to improve the status of Greek lakes, management measures to reduce eutrophication should be applied. Monitoring can further support the evaluation of the effectiveness of management measures.

# Reduced transfers of metals and metalloids contaminants from pelagic *Sargassum* spp. accumulated in artificial floating barrier device

Cipolloni, O-A.<sup>1\*</sup>, Simon-Bouhet, B.<sup>2</sup>, Couture, P.<sup>3</sup> & Pascal, P-Y.<sup>1</sup>

<sup>1</sup>Université des Antilles, Équipe Biologie de la mangrove, Institut de Systématique, Évolution, Biodiversité, ISYEB, UMR 7205, UFR SEN, 97100 Pointe-à-Pitre, France <sup>2</sup>LIENSs, Institut du littoral et de l'Environnement, CNRS-ULR, La Rochelle, France <sup>3</sup>Centre Eau Terre Environnement, INRS-ETE, Québec, Canada \*oceanne.cipolloni@gmail.com

Since 2011, holopelagic *Sargassum* are massively stranding in the coastal environments of the Caribbean Islands inducing damages for public health, coastal ecosystems an economy. To limit those risks, dams composed of floating buoys carrying dragging nets can be placed in front of sensitive areas to avoid *Sargassum* stranding. In order to evaluate the potential transfer of metallic trace element (MTE)

from *Sargassum* to adjacent marine life, phanerogam (*Halophila stipulacea, Thalassia testidinum*) and urchin (*Lytechinus variegatus*) were sampled close and far anti-*Sargassum* dams installed since December 2019 (Baie Cayol) and February 2019 (Cap-Est) in Martinique (French West Indies). In order to evaluate effects of long time stranding a bay without dams (Baie-Trésor) was sampled. Algae (*Dictyota spp.*) were placed close and far from accumulated *Sargassum* during four days in order to evaluate short term evolution of their contamination. All sampling was realized during two periods with active (July 2021) and reduced (January 2022) stranding of *Sargassum*. Measured concentrations of 28 metal(loid)s trace elements reveal *i*) an absence of MTE contamination spatially, close or far of dams whatever the site (BT, CA and CE) *ii*) an absence of MTE contamination temporally for the periods of 2021 and 2022 *iii*) higher concentration for the tAs in *Dictyota spp*. than the others marines' organisms analyzed.

## Canarian Harmful Algae Observatory: new trends in the study and monitoring of HABs in the Canary Islands, Spain

Soler Onís, E.<sup>1,2</sup>\*, Herrera Pérez, R.<sup>3</sup> & Ayza Mascarell, O.<sup>3</sup>

<sup>1</sup>Observatorio Canario de Algas Nocivas (OCHABs), FCPCT-ULPGC, Parque Científico Tecnológico Marino de Taliarte, 35214 Taliarte, Las Palmas, Canary Islands, Spain

<sup>2</sup>Grupo de Ecofisiología Marina (EOMAR), IU-ECOAQUA, Universidad de Las Palmas de Gran Canaria, Campus Universitario de Tafira, 35017, Las Palmas, Canary Islands, Spain

<sup>3</sup>Servicio de Biodiversidad, Viceconsejería de Medio Ambiente, Consejería de Agricultura, Ganadería, Pesca y Medio Ambiente, 35003, Las Palmas, Canary Islands, Spain

\*emiliosoleronis@fpct.ulpgc.es

In the last two decades, the marine environment around the Canary Islands, Spain has experienced environmental changes induced by climate change and human activity. These changes have caused the emergence, expansion, and growth of potentially harmful microalgae and cyanobacteria (HABS) on the coasts. To address this issue, the INTERREG MAC projects MIMAR and MIMAR+ (MAC/4.6d/066, MAC2/4.6d/249) aimed to create the Canarian Harmful Algae Observatory (OCHABs) in the Canary Islands. The OCHABs in the Canary Islands has the main tasks of monitoring and controlling the factors that determine the presence of HABS. This includes analysing the factors that determine the distribution of HAB species, their genetic variability, and the biodiversity of HAB communities. Additionally, the observatory studies the biotoxins produced by these microorganisms and their effects on the population. Between 2016 and 2023, 530 samplings in 91 locations across the Canary Islands resulted in 5,380 samples, identifying 742 microalgae and cyanobacteria species, including 45 harmful benthic genera, *Gambierdiscus, Ostreopsis, Coolia, Prorocentrum, Amphidinium* and *Lyngbya* that have a significant impact on the environment and human health (Ciguatera, DSP, Palitoxicosis and DTT).

The results of the observatory's work are presented, including the identification, monitoring, and control of the factors that determine the distribution of HABs species, potential trends associated with climate change, the biodiversity of potentially harmful microalgae communities, and the monitoring and control protocol of potentially harmful microalgae and cyanobacteria in the Canary Islands.

#### WORKSHOPS

## "Research on microphytobenthos: present advances and future challenges"

## Biofilms from the bottom up, how species attributes influence microphytobenthic ecosystem functioning

#### Underwood, G.J.C.

School of Life Sciences, University of Essex, U.K. gjcu@essex.ac.uk

Microphytobenthic biofilms consist of a diverse range of photosynthetic and heterotrophic species (cyanobacteria, diatoms, euglenids, e.g. various taxa of proteobacteria and Bacteriodetes). Though most measures of biofilm ecological function are made at the level of the "biofilm", these emergent properties are the result of all the different species physiologies and species-species interactions occurring within the biofilm matrix. The ability to maintain high rates of photosynthesis is due to a range of adaptions, including photophysiological flexibility, rapid induction of non-photochemical quenching, and behavioural movements to optimise light climate conditions. Some of these differences relate to different ecological contexts; e.g. exposed intertidal or shallow subtidal tropical sand and mudflat assemblages, sea ice assemblages. But different taxa within the same biofilm exhibit differing strategies, for example, with different species of benthic diatoms showing different diurnal and tidal migration patterns, light optima and light-sensing abilities, and photophysiology. We are also just beginning to discover evidence of close bacteria-algal species interactions (e.g., linked to carbon flow and growth). This combination of species diversity, photophysiological diversity, behavioural diversity and species-species interactions results in biofilms showing high resistance and resilience to environmental stresses, maintaining their ecological functioning across a very wide range of ecological conditions.

#### **Response of microphytobenthos to multiple stressors**

#### Paterson, D.M.

Scottish Oceans Institute, School of Biology, University of St Andrews d.paterson2st-andrews.ac.uk

There are strong reasons why the study of microphytobenthos (MPB) is becoming increasingly important. The coastal systems of the world are being rapidly and seriously affected by climate change, including sea level rise and storm frequency. In addition to climate effects, there is also the pervasive influence of point-source and dispersed pollution, such as pharmaceutical, pesticides and plastics which also affects these systems. The MPB are a diverse group of microbial autotrophs and their importance as primary producers is wellknown but they also support several other critical ecosystem services. An interesting feature of MPB assemblages is the number of species that co-exist and their ability partition the niche space available. This adaptability among species has made diatom assemblages extremely useful and interpreting past and examining present ecological conditions, using such methodologies as diatom stratigraphy, transfer functions and the trophic diatom index. Therefore, we recognise that the diatoms are adaptable and responsive to environmental change and as such their response to the

combined effects of climate and anthropogenic pollution is an area worthy of study. This presentation will consider the important role of MPB and the past and present research being conducted into multiple stressors effects on diatom assemblages and consideration of their resilience into the future.

#### The metabolites of light: untargeted metabolomic approaches bring new clues to understand light-driven adaptation of intertidal mudflat biofilm

Doose, C., Hubas, C.

MNHN UMR BOREA, France \*caroline.doose@mnhn.fr

The microphytobenthos (MPB) inhabiting marine sediments is a microbial community of primary producers playing a key role in shallow coastal environments and estuarine ecosystem functioning. The diatoms, brown microalgae usually dominating the MPB, can phase their behavior and metabolism to the strong intertidal light variability through the nonphotochemical quenching (NPQ), or by vertically migrating in the sediment. These lightadaptation mechanisms trigger a lot of scientific interest with numerous studies in literature, but the biological processes and metabolic mechanisms involved in light-driven vertical migration remain largely unknown.

In this study, we exposed a migratory biofilm of mudflat for 30 min to a light gradient from 50 to 1000  $\mu$ mol photons m<sup>-2</sup> s<sup>-1</sup> PAR. To explore the global metabolic responses to the irradiances we applied an untargeted metabolomic analysis method. After bioinformatic treatment, 96 compounds were found in CHCl<sub>3</sub> fractions and 142 in MeOH fractions. The samples exposed to HL (500, 750 and 1000  $\mu$ mol photons m<sup>-2</sup> s<sup>-1</sup> PAR) were essentially characterized by alka(e)nes with bacterial and diatom origin.

The oxylipins represent the best described signalling molecules in diatoms and they are generated from ROS reaction with polyunsaturated fatty acids (PUFAs). These compounds were also measured to investigate their possible implication in the microphytobenthic biofilm response to light. The oxylipins derived from the EPA significantly increased under HL compared to LL and ML. Since EPA is a diatom-related PUFA, this response could be attributed to these microalgae.

These results highlighted possible roles of alka(e)nes and oxylipins in MPB light acclimation.

## Phototobiology of a benthic diatom from the subtidal sediments of the bay of Brest

Isaia, A.<sup>1</sup>, Wahbi-Fusier, K.<sup>1</sup>, Breton, P.<sup>1</sup>, Kernalleguen, I.<sup>2</sup>, Delebecq, G.<sup>1</sup>, Leynaert, A.<sup>1</sup>, Heggarty, K.<sup>2</sup> & Lavaud, J.<sup>1</sup>

<sup>1</sup>LEMAR-Laboratory of Marine Environmental Sciences, Univ Brest, CNRS, IRD, Ifremer, LEMAR, F-29280 Plouzané, France <sup>2</sup>Optics Department, IMT Atlantique, F-29280 Plouzané, France anna.isaia@univ-brest.fr

Microphytobenthos (MPB) communities are responsible for an important fraction of the overall primary productivity in coastal ecosystems, particularly in estuarine regions such as the Bay of Brest. MPB is mainly composed of benthic motile diatoms which are found in intertidal and subtidal (< 10 m) sediments. Subtidal MPB, in contrast to intertidal MPB, has comparatively received much less attention, especially regarding the regulation of its photosynthetic productivity. Yet, its light environment is complex and characterized by low intensities and a spectrum 'enriched' in blue-green wavelengths.

#### WORKSHOPS

Recent studies have shown the crucial role of light quality, especially blue wavelengths, in the triggering and regulation of essential biological processes (cell division, photoacclimation, directed motility in sediments, etc.). The present study aims to better understand how a benthic diatom species, abundant in the subtidal sediments of the bay of Brest, namely *Pleurosigma strigosum*, responds to its light climate. To do so, *P. strigosum* was grown under different light conditions, including different light spectra and intensities: green-blue that reproduces 10 m depth, blue and green to isolate photophysiological responses to these wavelengths, and compared to a usual 'white' light spectrum. Growth, carbon and silicates content, pigments, cell-wall (frustule) structure, photosynthesis, photoprotection and some optical properties of the cells were monitored to determine the light-response of *P. strigosum*. The talk will show how *P. strigosum* is particularly well-adapted to low intensity and blue light wavelengths.

## Multi-scale temporal variability in MPB biomass and production in diatom- and euglenophyte dominated estuarine biofilms

Bas, D.A.<sup>1,2</sup>, Sabbe, K.<sup>2</sup>, van der Wal, D.<sup>3</sup>, Cox, T.<sup>1</sup> & Meire, P.<sup>1</sup>

<sup>1</sup>University of Antwerp, Department of Biology, ECOSPHERE, Belgium <sup>2</sup>Ghent University, Department of Biology, Protistology & Aquatic Ecology (PAE), Belgium <sup>3</sup>Royal Netherlands Institute of Sea Research (NIOZ), Department of Estuarine & Delta Systems (EDS), The Netherlands dorian.bas@uantwerpen.be

Microphytobenthos (MPB) biomass is characterized by pronounced temporal variability at multiple scales, in relation to tidal and diurnal, spring-neap, seasonal and interannual cycles. Field sampling but also airborne remote sensing is not able to fully capture this variability. We installed optical sensors to measure Normalized Difference Vegetation Index (NDVI) on estuarine intertidal flats at three locations (marine, brackish and freshwater) along the salinity gradient of the Schelde estuary, and monitored MPB surface biomass from October 2020 to september 2022 every ten minutes.

We found a similar correlation between NDVI and chlorophyll a in the freshwater and brackish intertidal flats. In the freshwater biofilms, which were dominated by euglenophytes, MPB biomass reached its highest values (NDVI of up to 0.69 corresponding to 260 mg Chl a/m<sup>2</sup>) during summer. In contrast, the brackish MPB communities were dominated by epipelic diatoms and showed a distinct biomass peak (NDVI up to 0.42 corresponding to 159 mg Chl a/m<sup>2</sup>) in winter (December-February, and to a lesser degree early spring). Both MPB communities showed rapidly fluctuating biomass during spring and summer. Vertical up- and downward migration during emersion was more gradual in the euglenophyte biofilms, but was very rapid in the diatom-dominated biofilms. In the marine station, MPB biomass was much lower, and showed little variability at all temporal scales. We will discuss the biomass patterns outlined above, and indicate how including high-resolution temporal variation in biomass can be used for benthic primary production modeling.

## Motility of epipelic diatoms: inter- and intra-specific variability of its photoprotective role under a realistic light microgradient

Morelle, J., Bastos, A, Frankenbach, S., Frommlet, J. & Serôdio, J.

CESAM – Centre for Environmental and Marine Studies and Department of Biology, University of Aveiro, Campus de Santiago, 3810-193 Aveiro, Portugal jerome.morelle@ua.pt Epipelic benthic diatoms are capable of directed motility, which has been hypothesized to be a major factor explaining their evolutionary success, diversification, and productivity. A long-standing idea is that they use motility as a form of photoregulation, to adjust their position in the photic zone, optimizing photosynthesis and avoiding excessive photoinhibitory irradiances. The fact that the sedimentary photic zone is very thin (1-3 mm) might be key to such a strategy, as cells can traverse these distances within a short time (< 30 min). However, due to methodological limitations in reproducing such light microgradients for experimentation, the degree to which diatom motility alleviates photoinhibition remained untested.

Here we present a new experimental setup that allows us to reproduce light gradients representative of those experienced by benthic diatoms living in exposed intertidal zones. By combining microfluidics and cellular bioimaging of chlorophyll fluorescence, we were able to track movement and photophysiology of various epipelic diatoms species in response to light microgradients.

The results show that motility can indeed be used to select optimal light levels and limit photodamage to the photosynthetic apparatus. However, the use of this form of photoregulation does not seem to be favored equally among different species, nor by all cells of the same population. This result highlights the inter- and intra-species variability regarding the ability to endure high light doses. Given the potential cost of motility, this raises the question of when motility is favored over other forms of photoprotection.

## Effect of heat stress on phototrophic biofilm photosynthesis in the context of climate change: a thermal dose approach

Laviale, M.<sup>1</sup>, Courcoul, C.<sup>2</sup>, Guyot, L.<sup>3</sup>, Baillard, V.<sup>1</sup>, Billoir, E.<sup>1</sup>, Boulêtreau, S.<sup>2</sup>, Leflaive, J.<sup>2</sup> & Jesus, B.<sup>3</sup>

<sup>1</sup>Université de Lorraine, CNRS, LIEC, F-57000 Metz, France

<sup>2</sup>Laboratoire écologie fonctionnelle et environnement, Université de Toulouse, CNRS, INPT, UPS, Toulouse, France

<sup>3</sup>Université de Nantes, Laboratoire Mer Molécules Santé, Nantes, France martin.laviale@univ-lorraine.fr

Climate change is threatening aquatic ecosystems worldwide, notably through the increase in heatwave intensity and frequency. Photosynthesis is one of the most heat-sensitive cellular processes, it is therefore necessary to better understand the heat stress effects on photosynthetic activity and corresponding primary productivity. Currently, these effects are not well documented due to the lack of a modeling approach able to integrate both temperature intensity and heat duration experienced during short extreme heat stress events. This is especially the case for phototrophic biofilms, for which the probability to face heat stress increases, in both fresh and marine ecosystems.

For this purpose, we implemented a laboratory controlled experimental set up (30 to 45°C, up to 3 h, using in vivo chlorophyll fluorescence as a proxy of photosynthetic activity) to expose to short-term heat stress benthic diatoms from shallow freshwaters or intertidal mudflats. The concept of "thermal dose" was then used to compute a dose of heat, defined as the product of time and an exponential function of temperature.

This approach allowed us to compare the tolerance and responsiveness to heat stress across a various set of experiments involving isolated algal strains, simplified synthetic communities or natural biofilms. We also showed that this modeling approach can be applied to in situ temperature data, for predicting the effects of heat stress events in situ. Our results show that even moderate heat stress can significantly impair primary productivity. While obtained on benthic diatoms, this approach offers promising research avenue for other (photosynthetic) organisms.

### "Getting together to fight the new invasive alien seaweed: Rugulopteryx okamurae (Dictyotales, Ochrophyta)"

## Rugulopteryx okamurae (Dictyotales, Ochrophyta): the first invasive alien seaweed of Union concern and what we know about it in Spain

Altamirano, M.<sup>1</sup>, De la Rosa, J.<sup>2</sup>, Carmona, R.<sup>3</sup>, Zanolla, M.<sup>1</sup>, Rosas-Guerrero, J.<sup>1</sup> & Román Muñoz, A.<sup>4</sup>

<sup>1</sup>Departamento de Botánica y Fisiología Vegetal, Facultad de Ciencias, Universidad de Málaga, Málaga, Spain

<sup>2</sup>Departamento de Botánica, Facultad de Ciencias, Universidad de Granada, Granada, Spain <sup>3</sup>Departamento de Ecología y Geología, Facultad de Ciencias, Universidad de Málaga, Málaga, Spain <sup>4</sup>Departamento de Zoología, Facultad de Ciencias, Universidad de Málaga, Málaga, Spain altamirano@uma.es

The Strait of Gibraltar was the site where an invasive Rugulopteryx okamurae event was first recorded in 2015, with Spain being the first country to suffer the environmental and socioeconomic impacts of this species. Since then, the species has expanded its distribution range both towards the Mediterranean and the Atlantic, which has led to its inclusion in the list of species of Union concern in 2021. So far, the species is present in Spain, Morocco, French Mediterranean coast, Southern Portugal, Azores, Madeira and Canary Islands, in chronological order of its expansion. Efficient management of the species requires a thorough knowledge of the species biology, ecology and interaction with native communities, as well as an economic estimate of its impact. This communication, which opens a workshop focused on R. okamurae, will review the response of the Spanish administration to the invasion of the species as well as the advances made to understand the high invasiveness of the species. Different research projects have focused on identifying the temporal and spatial invasiveness windows. Time of maximal invasiveness has been identified through the study of the vegetative and reproductive phenology of the species on two different communities, Posidonia oceanica meadow and a photophilous algae community, to identified moments of maximal recruitment and production. Spatial windows have been identified with distribution models at different scales. In addition, results on the socioeconomic impact of the species on the fishing sector and for the management of the affected beaches will be shown.

## Current status of the invasive brown alga *Rugulopteryx okamurae* on the Moroccan coast

El Aamri, F.<sup>1</sup>, Rami, M.<sup>2</sup> & Tamsouri, N.<sup>1</sup>

<sup>1</sup>National Institute of Fisheries Research,M'diq-Morocco <sup>2</sup>Faculty of Sciences of Tetouan (Abdelmalek Essaadi University), Morocco \*elaamri@inrh.ma

Seaweed invasions are known as one of the main threats to coastal resources and biological diversity. The brown alga *Rugulopteryx okamurae* is one of the newest non-indigenous example species. The invasive macroalga *R. okamurae* represents an unprecedented case of bioinvasion by marine macroalgae facing the Moroccan and European coasts. Since its first observation in Belyounech in 2016, the brown algae *R. okamurae* has invaded the rocky bottoms of our marine coasts (North Atlantic and a large part of the Mediterranean coast) from -0.15 m to -20 m deep (it has also been observed at -40 m). The

coverage of the brown algae in some sites exceeding 90%, accompanied by a significant change in the structure of benthic communities causing the physical displacement of native species due to substrate occupation and preventing the fixation of larvae or propagules of other species. *R. okamurae* not only has the potential to affect the ecological balance of the community, but also fundamental economic activities such as fishing and tourism.

## Following the invader *Rugulopteryx* okamurae in Azores (NE Atlantic): challenges and perspectives

Faria, J.<sup>1</sup>, Prestes, A.<sup>1</sup>, Rodrigues, A.<sup>1,2</sup>, Santos, A.<sup>1,2</sup>, Rodríguez, S.<sup>2</sup>, Cacabelos, E.<sup>3</sup>, Parente, M.I.<sup>1,2</sup>, Martins, G.M.<sup>1</sup> & Costa, A<sup>1,2</sup>.

<sup>1</sup>*CIBIO*, Research Center in Biodiversity and Genetic Resources, InBIO Associate Laboratory <sup>2</sup>*Faculty of Sciences and Technology, University of the Azores, Ponta Delgada, Portugal.* <sup>3</sup>*Hydrosphere - Environmental laboratory for the study of aquatic ecosystems, Vigo, Spain* joao.fo.santos@uac.pt

In European waters, the non-indigenous brown macroalga *Rugulopteryx okamurae* is the most recent example of an unprecedented case of bioinvasion by a marine organism, causing considerable ecological impacts on coastal communities. In 2020, the species was first recorded in the north-eastern Atlantic archipelago of the Azores close to one of the islands largest harbors (Ponta Delgada, São Miguel island), but presumably present since 2019. The alga quickly proliferated and over a very short period of time it became the dominant species covering most of the rocky bottom around the island and producing substantial accumulations of algal wrack on coastal areas. Native species were displaced, and invaded communities were structural different and supported a much more impoverished and homogenous assemblage. The species has also expanded to other islands where is now well-established. Here, we document the timeline of *R. okamurae* distribution across the archipelago, examining possible introduction and dispersion pathways. We also assess temporal changes in its life history characteristics and highlight its impacts on the structure of shallow water marine benthic communities. Finally, we discuss ongoing research and the challenges and perspectives associated with management strategies to monitor and mitigate the effects of *R. okamurae* on coastal ecosystems in the Azores.

## Monitoring and life cycle of *Rugulopteryx okamurae* in the NW Mediterranean Sea

Borriglione, M., Blanfuné, A., Ruitton, S. & Thibaut, T.

Aix Marseille University, Université de Toulon, CNRS, IRD, MIO (Mediterranean Institute of Oceanography), UM 110, 13288, Marseille, France marie.BORRIGLIONE@univ-amu.fr

The invasive *Rugulopteryx okamurae* (Dictyotales, Phaeophyceae), has been introduced to the Mediterranean Sea two decades ago. *Rugulopteryx okamurae* was first observed in 2002 in the Thau coastal lagoon (France), likely due to the import of Japanese oysters for aquaculture. The first Mediterranean report in the open sea dates back in 2013 at Agde (South of France). In 2015, the species was recorded on the both sides of the Strait of Gibraltar (Spain). Highly invasive, the species deeply impacted the algal communities of the Alboran Sea. In 2018, *R. okamurae* was found in the bay of Marseilles and colonized the subtidal rocky reef between 5- and 25-meters depth. The species is present along 60 km of coast in Marseilles and along 2 km of coast at Agde. Unlike the Alboran Sea, it

accumulates only in two beaches at Marseille (Callelongue and Marseilleveyre). The species can form dense monospecific population covering up to 100 % of the substrate. We studied the life cycle of the species from culture in aquarium and *in situ* with regular sampling. From the sporophytes, tetraspores and monospores are formed given fusiform germlings on the thallus. The germlings tend to separate in 45 days and get attached to the substrate thanks to rhizoid formations. On the thallus, from clusters of cells arise new seedlings at the same stage of the mother plant which get detached to be dispersed.

## Spread of the invasive brown alga *Rugulopteryx* okamurae in Portugal (NE Atlantic)

Liulea, S., Serrão, E.A. & Santos, R.

Center of Marine Sciences, University of Algarve, Campus of Gambelas, Portugal.

The brown seaweed *Rugulopteryx okamurae* was introduced in the Mediterranean in 2002, originating from the northwest Pacific, probably with oysters that were imported for aquaculture. In 2016 it was observed in both sides of the Strait of Gibraltar where the Mediterranean Sea meets the Atlantic Ocean, displaying invasive behaviour and developing massive amounts of biomass that accumulate on beaches causing high ecological and socioeconomic impacts. Here we report the first observations of *R. okamurae* in southwestern Portugal in 2020 and its impact on macroalgae species richness and abundance based on observations before (October 2020) and after (June 2021) the invasion and control sites. The large biomass accumulation of *R. okamurae* is having a profound negative effect on the macroalgae community, decreasing abundances of several species and causing the local loss of several other macroalgal species.

## Integrative approach assessing the invasion by *Rugulopteryx okamurae* in Madeira Island

Bernal-Ibáñez, A.<sup>1</sup>, Triay-Portella, R.<sup>1,2</sup>, Albert-Fonseca, A.<sup>1</sup>, Schäfer, S.<sup>1</sup>, Cacabelos, E.<sup>3</sup>, Gestoso, I.<sup>4</sup> & Canning-Clode, J.<sup>1,5</sup>

<sup>1</sup>MARE – Marine and Environmental Sciences Centre / ARNET - Aquatic Research Network, Agência Regional para o Desenvolvimento da Investigação, Tecnologia e Inovação (ARDITI), Portugal

<sup>2</sup>Grupo en Biodiversidad y Conservación (BIOCON), Instituto Universitario de Investigación en Acuicultura Sostenible y Ecosistemas Marinos (IU-ECOAQUA), Universidad de Las Palmas de Gran Canaria, Spain

<sup>3</sup>Hydrosphere-Environmental laboratory for the study of aquatic ecosystems, Vigo, Spain

<sup>4</sup>Department of Biology, Faculty of Marine and Environmental Sciences of University of Cádiz, Puerto Real, Spain

<sup>5</sup>Smithsonian Environmental Research Center, Edgewater, MD, United States alejandro.bernal@mare-centre.pt

After the arrival and proliferation of *Rugulopteryx okamurae* in the Açores archipelago, the spread of this species continues in the Macaronesian Region, being reported in the Madeira Archipelago in December 2021. The first risk assessment (AS-ISK) of this species in Madeira Island, developed in early 2022, showed a medium-high risk of becoming invasive in the current context and under future climate change scenarios. We performed a first mesocosm experiment to understand the performance of *R. okamurae* under grazing pressure during the first stages of the invasion process and different temperature regimes. Furthermore, we are assessing the invasion process in Madeira Island in an ongoing monitoring of benthic habitats since spring 2021 (before the arrival of *R. okamurae*). Through this, we are evaluating

changes in benthic communities (algal cover-functional traits, species diversity, sea-urchins density) and the potential effect of protected areas against the invasion. The description of the morpho-space occupied by *R. okamurae* as a function of the type of habitat it exploits, as well as the interaction with other nonnative algae, is underway. Finally, we applied ecological niche modeling and habitat suitability projections to assess potential niches, the probability of colonizing new environments and its expansion/contraction in future scenarios. Through all these methods and techniques, we are assessing the most important marine bioinvasion reported up-to-date in Madeira, and probably in Macaronesia, which is already producing a phase shift in coastal benthic systems from barren-states dominated areas to monopolized habitats by *R. okamurae*.

## Impacts of the early spread of *Rugulopterix okamurae* in the Canary Islands

Haroun, R.<sup>1</sup>, Nadermann, J.<sup>1</sup>, Correa Falcón, S.<sup>1</sup>, Valdazo, J.<sup>1&2</sup>, Hachero Cruzado, I.<sup>3,4</sup>, Triay-Portella, R.<sup>1</sup> & Manchado, M.<sup>3,4</sup>

<sup>1</sup>Biodiversity & Conservation Research Group, IU-ECOAQUA, Scientific & Technological Marine Park, Univ. of Las Palmas de Gran Canaria, Crta. Taliarte s/n 35214 Telde, Spain. <sup>2</sup>Elittoral Environmental Consulting Co., Technological Park of the ULPGC, Edif. Polivalente II, C/ Practicante Ignacio Rodríguez, s/n Campus de Tafira, 35017, Las Palmas, Spain

<sup>3</sup>IFAPA Centro El Toruño, Junta de Andalucia, Camino Tiro Pichon s/n, 11500 El Puerto de Santa Maria, Cádiz, Spain

4*Crecimiento Azul, Centro IFAPA El Toruño Unidad Asociada al CSIC, Spain* ricardo.haroun@ulpgc.es

In the month of June 2022, the first individuals of the brown macroalgae *Rugulopterix okamurae* (Dawson) I.K. Hwang *et al.* were collected in the shoreline of Gran Canaria Island. The first populations were located at the rocky coasts south of the main harbour of the Canary Islands. Since that time, new populations have been identified along the shorelines of Gran Canaria and, more recently, also in the nearby Fuerteventura Island. The arrival of this invasive brown seaweed has produced significant changes in the structure and composition of the benthic assemblages, with a drastic reduction of autochthonous macroalgal species. Besides its ecological threats to the indigenous marine flora, diverse social and economic impacts are being reported with increasing detrimental effects.

This contribution will describe the arrival and dispersion mechanism of *R. okamurae* along the Canary Islands as well as the ecological, social and economic impacts, which are mainly related with the dramatic biomass production of this alien species.

### **Index of Authors**

### Α

Aakre, I. 85 Abellán, E. 337 Abomohra, A. 76 Aboudagga, C. 132 Aboussalam, S. 339 Abram, M. 269 Abreu, M.H. 237 Accoroni, S. 93 Achterberg, E.P. 273 Adamska, D. 269 Adler, L. 125 Afonso-Carrillo, J. 206, 286 Agraso-Martínez, M.M. 333 Aiese Cigliano, R. 65 Aires, T. 64 Aksoy, B. 305 Alban, C. 260 Albers, S. 311 Albert-Fonseca, A. 350 Alcolombri, U. 114 Alevizos, E. 76 Alexiadou, D. 164 AlgaeTraits editors 210 Al-Handal, A.Y. 307 Allahverdiyeva-Rinne, Υ. 171, 268, 269 Allen, A. 61, 232 Allen, R. 304, 311 Almeida, N. 211 Almeida, S. 72 Alongi, G. 74 Altamirano, M. 73, 339, 348 Álvarez-Canali, D. 44, 206, 286 Alvarez-Roa, C. 70 Alverson, A.J. 145 Amano, R. 126 Ambar Prabowo, D. 98

Amin, S.A. 151 Amsler, C.D. 158, 193 Añasco, B. 240 Andersen, R.A. 48 Andersen, S. 298 Andersen, T. 216, 281, 301 André, J. 119 Anesio, A.M. 295, 299 Angarita, L. 87 Angulo, J. 176 Annachi, A. 287 Anguet, A. 340 Antipova, C.G. 172, 330 Antreich, S.J. 105 Antonaroli, S. 324 Ar Gall, E.<sup>†</sup> 280 Arakaki, N. 201 Arapov, J. 210, 212, 225 Arason, S. 314 Archontikis, O. 141 Arenas, F. 95, 118, 290 Arendt, D. 159 Arnaldo, M.D.G. 58, 310 Arnosti, C. 164 Arnould-Pétré, M. 44 Aroui Boukbida, H. 277, 278 Arriaga, O. 120 Arvanitidou, C. 152, 244 Arz, H.W. 92 Asnaghi, V. 117 Asselman, J. 262 Assis, J. 72, 73, 159 Assunção, M.F.G 305 Atteia, A. 215 Audo, G. 79 Audoor, S. 156 Avouac, A. 237, 238 Avrahami, Y. 97, 153 Aydlett, M. 237

Aymerich, T. 306 Ayres, L. 147 Ayza Mascarell, O. 343

### B

Babenko, I. 111 Babin, M. 196, 267 Badis, Y. 62, 237, 240 Baillard, V. 130, 347 Bailleul, B. 110, 132, 134, 176, 187, 266 Bailón, R. 334 Bajjouk, T. 280 Bakrač, A. 227 Balde, B.S. 278 Baldermann, S. 203 Ballesta, L. 47, 218 Ballesteros, J.C. 245, 334 Ballottari, M. 82, 211 Bañares-España, E. 128, 285 Bárbara, I. 99, 218 Barbeyron, T. 231, 242 Barcytė, D. 41 Barda-Chatain, M. Baričević A. 202, 215 Barillé, L. 76, 166, 221 Barone, M.E. 81 Barreiro, R. 218, 275 Barrera-Redondo, J. 147, 155 Barreto, L.M. 64, 99 Barrett, J. 257 Barrientos, S. 218, 275 Barros, A.I.R.N.A. 323 Barros-Barreto, M.B. 47 Barrow, C.J. 85 Bártolo, V. 130 Bartsch, I. 99, 123, 128, 147, 171, 192, 194, 216, 226, 296 Bas, D.A. 264, 346

Bastos, A 131, 346 Baud, A. 146 Baudoux, A.-C. 55 Baulina, O.I. 81 Bayer, T. 294 Bear, B. 211 Beardall, J. 263 Beaulier, C. 260 Bec, B. 215 Becerro, M.A. 122 Bedoux, G. 79, 235, 321 Beeckman, T. 153 Behnke, J. 161 Bekkby, T. 159 Belcour, A. 313 Belisova, D. 156 Bellamoli, F. 82, 211 Bellgrove, A. 83, 320 Bělohlav, V. 297 Belshaw, N. 185 Benavides Lahnstein, A.I. 334 Benavides, M. 291 Bendif, E.M. 141 Benning, L.G. 191, 295, 299 Ben-Sheleg, A. 314 Bérard, J-B. 240 Berglin, M. 319 Berline, L. 87, 291 Bermejo, R. 333 Bernal-Ibáñez, A. 73, 350 Bernard, O. 322 Berrendero Gómez, E. 307 Berrin, J-G. 138 Bertrand-Michel J. 181 Betterle, N. 82 Bevilacqua, S. 74 Béziat, P. 114 Biancacci, C. 83 Bianchelli, S. 287 Bidault, A. 205 Bihannic, K. 90

Bilajac A. 74, 276, 282, 293 Bilcke, G. 153, 156, 250, 262 Billoir, E. 130 Billoud, B. 152 Bionaz, M. 294 Bischof, K. 116, 186, 192, 194, 226, 272, 298 Bišová, K. 256 Bizien, A. 94, 340 Bjornson, S. 140 Blais, C. 119 Blanc, G. 235 Blanc-Mathieu, R. 232 Blanco González, M. 227 Blanfuné, A. 207, 276, 348 Blaszczyk, A. 307 Blatt, M. 125 Blaza, J. 257 Bligh, M. 162, 192 Blond, A. 115 Boada, J. 282 Bobanović-Ćolić, S. 289 Boderskov, T. 167 Boehme, T. 293 Bogaert, K.A. 153, 154, 252 Bokov, M. 109, 245 Bolchi, G. 212 Bolhuis, H. 57 Bolius, S. 92 Bolívar, F.C. 245, 334, 335 Bolívar, M. 245, 335 Bolton, J.J. 99 Bon, C. 145 Bonačić, T. 210, 212 Bonesteve, A. 278 Bonnefond, J-M. 162 Bonthond G. 158, 294 Bonzom-Audiffrin, C. 85 Borchert, E. 63 Borer, E.T. 113 Borer, G. 97 Borhan, E. 325

Borriglione, M. 349 Bosch, N. 227 Boscq, S. 152 Boucher, C. 119 Bouchet, S. 114 Boudesocque-Delaye, L. 310 C.F. Boudouresque, 276 Bougaran, G. 240 Bouguerba-Collin, A. 102, 231 Boulêtreau, S. 130, 347 Boulho, R. 79 Bouly, J.-P. 134, 187 Bouma, T. 275 Bourguet-Kondracki, M-L. 115 Bourgougnon N. 56, 77, 79, 235, 241, 308, 321, 327 Bourguignon, J. 260 Bousquet, L. 153 Bousso, N.C. 203, 277, 278 Bowler, C. 121, 141, 144, 187, 229, 252 Bowles, A. 140, 190 Boy, V. 77 Boyé, A. 94 Boyen, C. 67 Brakel, J. 46, 93, 212, 216 Braglia, R. 328 Brand, M. 194 Branscombe, L. 61 Bråtelund, S. 146 Brawley, S.H. 237 Brébion, J. 317 Brehmer, P. 203, 212, 277, 278 Breton, P. 345 Briand, E. 149 Bridle, A.R. 263 Brillet-Guéguen, L. 228, 229, 231 Bringloe, T.T. 140, 224 Britton, D. 263

Broadwell, E.L.M. 190, 295 Brodie, J. 44, 46, 93, 184, 189, 208, 237, 334 Brotas, V. 166 Broudin, C. 72 Brownlee, C. 108 Brück, T. 261 Bruckner, C. 179 Bruhn, A. 167, 182 Brun, F.G. 198 Brunet, M. 242 Bruni, L. 234 Bruno, L. 324, 328 Bruyant, F. 196 Buccarello, L. 324 Buck-Wiese, H. 162, 192 Budziak, M. 338 Buerger, P. 70 Buffoli, M. 100 Bulić, A. 212 Bura, R. 245 Burel, L. 308 Burel, T. 94, 205, 280, 340 Burgunter-Delamare, B. 67 Burlot, A-S. 235 Burokienė, D. 287 Burrows, M.T. 159 Burton J.-A. 71 Bury, H. 311 Buschbaum, C. 116 Buschmann, A.H. 127, 193 Bušelić, I. 210, 212 Butler, J. 174 Bužančić, M. 210, 225

### С

Cacabelos, E. 349, 350 Cadier, M. 90 Cadieu, S. 235 Caetano, N.S. 309 Calchera, A. 139 Calder, G. 258 Calgaro, M. 211 Calvo, I. 245 Camacho, O. 201 Campbell, D. 190 Campion, E. 81 Camplong, C. 55 Camus, C. 65 Cañete, S. 192, 297 Canning-Clode, J. 73, 350 Cano Cejas, I. 313 Cantonati, M. 211 Canvin, M.C. 265 Capistrant-Fossa, K.A. 237 Capp, S. 256 Car, A. 288, 289 Cardador, M. 107 Cardoso, H. 170 Cardoso, P.G. 290 Carlson, D.F. 159 Carmona, R. 192, 297, 339, 348 Carradec, O. 69 Carrano, C.J. 103 Carreño, E. 336 Carrier, G. 240 Carvalho, W.F. 285 Casabianca, S. 212 Casamatta, D.A. 207 Castagnone-Sereno, P. 310 Castaldi, A. 115 Castellani, G. 195 Cazzaniga, S. 211 Cebrian, E. 282, 287 Cébron, A. 60 Cecchi, P. 291 Čepukoit, D. 287 Cérantola, S. 83 Chaerle, P. 56, 106, 174, 250 Chaffron, S. 54 Chan, W.Y. 70 Chandola, U. 54, 234 Changeux, T. 87, 291

Chapman, A. 179 Chapuis, L. 63 Charrier, A. 240 Charrier, B. 152 Charton, F. 141, 252 Châtelain, B. 214, 278 Chaumier, T. 54, 67, 234, 253 Chaux, F. 246 Cheloni, G. 165 Chevalier, F. 58, 61 Chevenier, A. 253 Chiantore, M. 115 Chiarore, A. 287 Chiou, T.-H. 322 Chiou, Y.-S. 322 Chiurazzi, M. 110 Chivkunova O.B. 81, 172, 330 Cho, T.O. 201 Choi, H.I. 260 Choi, Y.J. 247 Choquet, Y. 132 Choulot, M. 308 Chovancek, E. 325 Chowdhury, S. 259 Christiansen, E.R. 179 Christiansen, F. 167 Christodoulou, A. 164 Ciftci, O. 143, 295 Cipolloni, O-A. 279, 342 Cisternas, S. 85 Čižmek, H. 273 Claquin, P. 177, 219 Clijsen, E. 113 Cloetens, P. 103 Cobos, P. 279 Cock, J.M. 62, 102, 143, 154, 228, 231, 247, 313 Coe, L.S.Y. 149 Coelho, S.M. 147, 153, 155, 228, 247, 289 Cojoc, L.C. 64

Čolić, B. 273 Colin, S. 202, 247 Collén, P.N. 310 Colletti, A. 287 Collin, R. 201 Coluccelli, A. 93 Comeau, S. 287 Compadre, A. 85 Concepción, Y. 286 Congestri, R. 84, 324, 328 Conlan, X. 83 Connan, S. 49, 87, 214, 278, 291, 308, 312, 317, 323 Contaldo, A. 324 Contreras, Y.R. 334, 335 Corrales, M.B. 204 Corre, E. 145, 228, 229, 231 Correa Falcón, S. 351 Corrêa, P.S. 309 Cosquer, N. 87 Cossard, G. 147, 289 Costa, A, 349 Couallier E. 78 Coudret, J. 72 Coulombier N. 270, 315 Courant, F. 165 Courcoul, C. 130, 347 Courtois, F. 176 Courtot, E. 310 Couture, P. 279, 342 Cox, T. 264, 346 Crec'hriou, R. 72 Cremades J. 59, 235, 242, 265 Critchley, A. 332 Croteau, D. 110, 134, 176, 266 Cruaud, S. 154 Cruz de Carvalho, H. 229, 252, 355 Cuberos, L.M. 245, 334, 335 Cunliffe, M. 61 Curien, G. 176

Cvetkovska, M. 194 Cvitković, I. 219 Cvjetan, S. 219 Czerwik-Marcinkowska, J. 205 Czjzek, M. 121, 238

### D

D'Alelio, D. 102 Dacks, J.B. 144 Daguin-Thiébaut, C. 72 Daher, A. 325 Dal Grande, F. 138 Dallet, R. 228 Dalmia, A. 254 Damsgård, B. 298 Dannay, M. 260 Danovaro, R. 287 Daroch, M. 43 Dartevelle, L. 154 Davalle-Corre, K. 312 Davey, M.P. 311 Davoult, D. 72 de Caralt, S. 282 De Clerck, O. 54, 73, 99, 138, 153, 206, 243, 252 De Cock, L. 262 de la Hoz, C.F. 167, 222 De la Rosa Alamos, J. 72, 334, 339, 348 De Luca, D. 217 de Oliveira, S.R. 191 de Vargas, C. 55 De Veylder, L. 156 Debit, A. 141, 229, 252 Deborde, J. 162 Decelle, J. 58, 61, 153, 232, 336 DeForest J. 158 Dehel Gamage, N.D. 58, 310 del Campo, J. 227 Del Olmo G. 59, 234, 235, 242, 265

Delage, L. 62 Delaux, P-M. 138 Delebecq, G. 345 Dellwig, O. 92 Del-Pilar-Ruso, Y. 337 Delpont, W. 311 Delva, S. 73 Demoulinger, G. 236 Deslandes, E. 87 Despalatović, M. 219 Destombe, C. 40, 95, 123, 145, 146, 147 Deter, J. 47, 218 Devime, F. 260 Devreese, B. 262 D'hondt, S. 156 Di Cave, A. 324, 328 Di Costanzo, F. 65, 83 Di Dato, V. 65, 83 Diadhiou, H.D. 277, 278 Díaz-Tapia, P. 50, 51 Dickenson, J. 108 Diedhiou, F. 212, 277, 278 Diehl, N. 192 Díez, I. 120 Dillehay, T.D. 145 Diop, M.S. 203, 212, 278 Diruit, W. 280 Dittami, S.M. 53, 67, 229, 313 Dobson, A. 319 Dominguez Almela, V. 197 Donoghue, P.J.C. 140, 190 Doose, C. 345 Dörflinger, G. 164 Dorrell, R.G. 121, 144 Dowle, A.A. 110, 255, 258 Dowson, M. 255, 258 Dromard, C. 87, 278 Dronne, R. 308 Drost, A.M. 281 Drysdale, C. 304, 311

Du, G. 166 Duarte, B. 179 Duarte, C.M. 159 Duarte-Neto, P. 291 Dubreuil, C. 85 Duc, C. 230, 233, 259 Duchêne, C. 187 Dufay, M. 123 Dufresne, F. 91 Dunker, S. 107, 336 Dupčić Radić, I. 288, 289 Dupont, G. 213 Durand T. 181 Durlave, R. 180 Dürselen, C.-D. 336 Düsedau, L. 194, 296 Dutz, J. 113 Dvořák, P. 43, 207

### E

Eckels, R. 268 Edvardsen, B. 69, 216, 281, 301 Edwards C. 173 Edwards, M.D. 185 Egan, S. 55, 235, 242 Egge, E. 216 Ehrenhaus, C. 201 Eichner, M. 133 Eikrem, W. 216, 281 Eiler, A. 216, El Aamri, F. 348 Eliáš, M. 41, 142 Elkalay, K. 339 Elmer, F. 161 Elster, J. 297 Emran, M. 79 Engelen, A.H. 63, 64, 179, 237 Engelmann, J. 57 Epp, L. 92 Epperlein, P. 147

Ergon, Å. 85, 146, 248 Escobar, M.C. 85 Estoup, P. 237

### F

Fabbrizzi, E. 287 Fahmi, V. 98 Fainsod, S. 153 Faisan, J.Jr. 93 Faith, M. 101 Falace, A. 74 Falcao, B. 66 Falciatore, A. 110, 132, 134, 137, 187 Falco P.P. 95 Falconet, D. 105 Fanesi, A. 322, 328 Fanuel, M. 121, 238 Faria, J. 349 Farnelid, H. 284 Faugeron, S. 65, 72, 145 Faye, M. 277, 278 Faye, S. 278 Feiters, M.C. 103 Félix, C. 165, 238 Feord, H.K. 191 Fernandes, I. 323 Fernandes, T. 323 Fernández, A.N. 128 Fernández, M. 73 Fernández, P.A. 127, 193 Fernández-Méndez, M. 161 Fernández-Torquemada, Y. 337 Fernie, A.R. 126 Ferrante, M.I. 100, 110, 157 Ferreira, P.C.G. 49 Ferté, S. 176 Ficko-Blean, E. 121, 238, 253 Field, J. 304, 311 Filatov, D. 141

Filbee-Dexter, K. 72, 159 Fillet, S. 245 Finazzi, G. 176 Fischer, P. 194 Flander-Putrle, V. 164 Fleury, G. 85 Flora, E. 330 Flores-Moya, A. 128, 285 Flori, S. 108 Fon, M. 281 Fontana, A. 176, 266 Force, A. 51 Fort, A. 185, 249 Fortič, A. 225 Fouilland, E. 165, 238 Frada, M.J. 97, 153 Fragkopoulos, E. 72 Francé, J. 164, 225 Francis, P. 71 Franco, J.N. 99 Frank, E. 299 Franke, K. 128 Frankenbach, S. 130n 131, 346 Fraschetti, S. 287 Frassine, D. 328 Fraumont, L. 146 Fredericq, S. 203, 341 Fredriksen, S. 194, 224, 296 Freile-Pelegrín, Y. 291 Freiler, M. 230 Frenken, T. 113 Freshwater, D.W. 201 Fricke, A. 203, 212, 278 Friedrich, B.M. 111 Frommlet, J. 131, 346 Fumagalli, P. 108 Füssy, Z. 61, 144, 232

### G

Gabriel, D. 201, 341 Gabrielsen, T.M. 224

Gachenot, M. 249 Gachon, C.M.M. 46, 93, 186, 237, 240 Gaikwad, T. 109 Gain, G. 176, 266 Gal, A. 106 Galica, T. 66 Galindo-De Santiago, M.C. 334 Galobart, C. 282 Garayev E. 270 Garbe, D. 261 García Abad, L. 307 García, J. 307 García-Cardesín, E. 265 García-Gómez, M.E. 152 García-González, M. 152, 244 García-Sánchez, M.J. 128, 285 Gardner, C.J. 337 Garnier, M. 149, 240 Garvetto, A. 186 Garzia, A. 93 Gasparotto, E. 82 Gast, R. 58 Gattuso, J-P. 159 Gauci, C. 171, 179 Gaudin, M. 54 Gauthier, O. 94 Gautier, M. 278 Gauyat, C. 312 Gavin, T.H. 110 Gavio, B. 204 Geairon, P. 162 Génin, A. 213 Gerber, T. 157 Gérikas Ribeiro, C. 190 Gernez, P. 90, 221 Gernigon, V. 237 Gerotto, C. 267 Gervois, A. 235 Gesson, M. 160, 272

Gestoso, I. 350 Ghobara, M. 110 Ghobrial, S. 162 Gianaroli, C. 215 Gifuni, I. 78 Gilic, M. 108 Gillon, P. 78 Giossi, C.E. 131 Giraud, M. 162 Girr, P. 110, 125 Gljušćić E. 74, 276, 282, 293 Gloaguen, N. 278 Gobert, T. 214 Gobet, A. 217, 237, 238 Godfroy, O. 147, 154, 155, 228, 229, 247 Godrijan, J. 246 Goecke, F. 85, 146, 224, 248 Gómez Garreta, A. 248 Gomez, E. 165 Gómez-Pinchetti, J.L. 335 Goncalves, O. 316 Gonthier, E. 72 González Castelli, E. 72 González, J. 120 Gonzalez, M. 72 Góral, T. 269 Gordillo, F.J.L. 192, 279, 297 Gordo, E. 192, 297 Gorelova, O.A. 81 Gorostiaga, J.M. 120 Goss, R. 107 Gosselin, M. 196 Gostyńska, J. 283 Got, J. 313 Goulding, D. 303 Gourvil, P. 190 Græsdal Rasmussen, T. 275 Graiff, A. 128 Gravendeel, B. 143 Green, D. 311

Greff, S. 79, 99 Grigoriev, T.E. 172 Grisel, S. 138 Grižančić, L. 202, 215 Grizon, J. 162 Groben, R. 57 Groisillier, A. 234, 248, 259 Gröndahl, F. 199 Grossmann, C.H. 195 Grundy, A.C. 58 Gudjonsdóttir, M. 314 Guée, L. 238 Guégnard, F. 310 Guérin, N. 69 Guérin, S. 196, 267 Guerquin-Kern, J-L. 60 Guihéneuf, F. 238 Guillemin, M.L. 63, 123, 145, 147, 231 Guillot, T. 214 Guillou, L. 162, 176 Guillouche, C. 235 Guinda, X. 167, 222 Guiry, M.D.G. 51 Gulbinas, Z. 338 Gundersen, H. 159 Gurgel, C.F. 201 Gustafsson, C. 162 Guyot, L. 130, 347

### н

Haas, F.B. 155, 289 Habchi, K. 249 Hachero Cruzado, I. 351 Hafner, D. 288, 289 Hakoun, E. 312 Hall, A. 208 Hällfors, H. 283 Hamilton IV, W. 158 Hamon-Giraud, P. 62, 313 Hancke, K. 159 Hanelt, D. 76

Hania, A. 133 Hansen, P.J. 119 Haon, M. 138 Hardouin, K. 79 Harnay, P. 294 Haroun, R. 73, 227, 351 Harrison, E.L. 109 Hartnell, D.M. 313 Hays, C.G. 158 Heagy, MK. 49 Heesch, S. 204 Hegaret, H. 285 Heggarty, K. 345 Hehemann, J.-H. 162, 192 Heintze, C. 111 Hejduková, E. 299 Helias, M. 94, 205, 280, 340 Hellige, I. 162 Helliwell, K. 61, 108, 109 Hennequart, F. 317 Hérault, J. 263 Herbert, H. 81 Herbette G. 270 Herburger, K. 102, 105 Hernández, I. 198, 333 Hernández, S. 227 Hernández-Chan, S. 290 Hernandez-Fariñas, T. 168 Herrera Pérez, R. 345 Hervat, M. 202 Hervé, C. 102, 231 Hildebrandt, L. 298 Hill, G. 171, 179 Hittorf, M. 186 Hoarau, G. 171, 237 Hoarau-Heemstra, H. 179 Hodač, L. 336 Hodoš, M. 109, 245 Hofmann, L.C. 216 Høgslund, S. 167 Holding, J. 167 Holst, N. 167

Holzinger, A. 105 Hommersand, M.H. 47 Hong, C.Y. 255 Hopes, A. 185 Hoppe, C.J.M. 89, 300 Hoshino, M. 289 Hotchkiss, S. 319, 326 Houbin, C. 72 Howe, C.J. 144 Hrólfsdóttir, A.Þ. 314 Hrouzek, P. 66 Htet, H.Y. 254, 314 Hubáček, M. 268 Hubas, C. 345 Huchette, S. 235 Hughey, J.R. 51 Huisman, J. 281 Huisman, J.M. 206 Hupp, J. 268 Hurd, C.L. 193, 263 Husa, V. 224

Iglesias, P. 333 Iha, C. 203 Im, S.H. 111 Inaba, M. 126, 185, 303 Indart, A. 64 Iñiguez, C. 192, 279, 297 Inomata, E. 274 Irit, D. 86 Isaia, A. 315, 345 Israel, Á. 223 Israelievitch, E. 176 Ito, S. 126 Iveša L. 74, 276, 282, 293 Izabel, G. 177

### J

Jackson, S. 319 Jacob J.F. 170 Jacqueline, F. 177 Jaeger, C. 299 Jahodářová, E. 207 Jakob, T. 107 Jam, M. 121 Jameson, I. 304 Jamroszczyk, M. 336 Janßen, M. 341 Jaramillo, M.A. 47 Jardim, B. 323 Jasprica, N. 289 Jaubert, M. 134, 187 Jauffrais T. 270, 315 Jauzein C. 116 Jensen, E.L. 132 Jesus, B. 130, 162, 234, 271, 347 Jiang, Y. 43 Jin, C. 316, 321 Jirout, T. 297 Joanne, P. 93 Joemmanbacks, T. 152 John, D.M. 208 John, U. 89, 100 Johnson, E.E. 237 Johnson, M.P. 118 Jokel, M. 325 Jónsdóttir, R. 319 Jossart, Q. 51 Jouanneau, D. 121, 170 Jouanno, J. 291 Jouneau, P.H. 58, 105 Juanes, J.A. 167, 222, 251 Juery, C. 58, 61, 232 Jueterbock, A.O. 99, 143, 171, 179, 186

### K

Kaiser, J. 92 Kajee, M. 162 Kaleb, S. 74 Kaleli, A. 288 Kambey, C.S.B.

Kammers, K. 113 Kane, C. 203, 277, 278 Karez, R. 183, 226, 275 Kargul, J. 269 Karosienė, J. 287, 338 Karsten, U. 92, 122, 128, 194, 341 Karthick, P. 332 Kasperoviciene, J. 338 Kaštovský, J. 42 Katsaros, C. 103 Katsiapi, M. 164 Kayacan, Y. 56 Kazakis, N.A. 330 Kefalas, G. 287 Keil, L. 261 Keller, J. 138 Kemel, S. 316 Kemitzoglou, D. 342 Kergosien, N. 87, 317 Kernalleguen, I. 345 Kervarec, N. 83 Keusching, C. 295 Khalil, K. 339 Khatei, A. 143, 179 Kholssi, R. 291 Khozin Goldberg, I. 80, 254, 314 Kihika, J. 174 Kim, G.H. 104, 156, 241, 244, 247, 250, 255, 261, 329 Kim, H.-S. 187, 260, 317 Kim, J.W. 187, 260 Kim, R. 329 Kim, R-W. 261 Kim, S.M. 318 Kim, S.Y. 250 Kim, Y. 261 Kirchmair, M. 186 Kittle, R. 201 Klein, C. 90 Klein, J. 291

Kleiner, F. 108 Klemetsdal G. 146 Kline, S. 216 Kloareg, B. 103 Klonowski, A.M. 57 Knjaz, M. 202, 215 Knoll, A.H. 106 Kociolek, J.P. 41 Kogovšek, T. 215 Konar, B. 72 Kooistra, W.H.C.F. 217 Koplovitz, G. 153 Koreivienė, J. 287, 338 Kotzabasis, K. 330 Kourist, R. 270 Kovač, Ž. 160 Kozlíková, E. 66 Kraan, S. 200 Kraffe, E. 312 Kranzler, C. 65 Krause-Jensen, D. 72,159 Kravtsov, A. 86 Krayesky, D. 201 Krayesky-Self, S. 201 Krela, R. 256 Kremp, A. 92, 113, 283 Kröger, N. 111 Kroneck, P.M.H. 103 Kroth, P.G. 111, 129, 135 Krueger-Hadfield, S.A. 147, 158, 229, 294 Krüger, S. 107 Krumhansl, K.A. 159 Krztoń, W. 338 Kumar Dolui, A. 254 Kumari, P. 103 Künzel, S. 294 Küpper, F.C. 103 Küpper, H. 103 Kurtoglu, E. 259 Kuwae, T. 159 Kvíderová, J. 297

### L

Labeille, M. 205 Labry, C. 116 Lachanidou, G. 168 Łaciak, M. 338 Lacour, T. 54, 90, 119, 135 Laeseke, P. 192 Lafferty, S. 311 Lagarde, F. 215 Lagunes, M-J. 291 Laiti, C. 211 Lajili, S. 308 Lalegerie, F. 321 Lam Kim, F. 83, 320 Lamaud, E. 162 Langer, S. 71 Lanoisellé, J.-L. 77, 327 Lanzirotti, T. 103 Larocque, R. 121 Larson, T.R. 71 Lasbleiz, M. 292 Lasserre, B. 235 Latella, L. 211 Latire, T. 321 Lau, J.S. 110, 125 Laugier, J. 67 Launeau, P. 162 Lavaud, J. 162, 196, 265, 345 Lavaud, R. 291 Lavaut, E. 123, 147 Lavenant, L. 78 Laviale, M. 130, 347 Lawton L. 173 Lbouhmadi, R. 239 Le Bars, A. 228, 229 Le Blay, G. 56, 241 Le Bras, Z. 114 Le Bris, A. 280 Le Corguillé, G. 145, 228 Le Dean, L. 270, 315 Le Duff, M. 94, 340

Le Duff, N. 60, 242 Le Floc'h, E. 165, 238 Le Gac, M. 95, 116, 155 Le Gall, L. 44, 47, 51, 207, 218, 220, 224 Le Gall, T. 72 Le Garrec, V. 340 Le Grand, F. 312 Le Guillard, C. 236, 308 Le Loc'h, F. 291 Le Moigne, F.A.C. 272 Le Moyec, L. 115 Le Panse, S. 249 Le Pennec, G. 115 Le Strat, Y. 241 Le, T.T. 260 Lebeau, T 58, 310 Lebiedzińska-Arciszewska, M. 269 Leblanc, C. 53, 62 Leboulanger, C. 165, 238 Lebouvier N. 270 Lebrun, M. 241 Leclerc, J.-C. 72 Lee, E. 302, 304 Lee, H-G. 316, 321 Lee, M-W. 321 Lee, Y.J. 260 Leflaive, J. 130, 347 Lefoulon, C. 125 Legeay, E. 53, 60 Legrand, C. 284 Lehtinen, S. 283 Leira, M. 218 LeKieffre, C. 58 Leliaert, F. 45, 138, 140, 206 Lemée, R. 115 Lemoine, M. 219 Lendormi, T. 327 Lenhof, O. 310 Lepen Pleić, I. 212 Lepetit B. 111, 129, 135

Leroux, C. 60, 62 Lesgourgues, M. 321 Leska, B. 338 Lesmes, U. 86 Lett, C. 291 Levain, A. 198 Levasseur M. 49 Lewis, M. 190 Leya, T. 295 Leynaert, A. 345 Li, N. 85 Li, X.D. 85 Li, Y.Q. 91 Libourel, C. 138 Liesner, D. 155 Lim, P.E. 46 Lima, F.P. 95, 96, 97, 118, 290 Lima, T. 305 Lin, L.-C. 322 Lin, S.-M. 206, 322 Linares-Maurizi A. 181 Lindehoff, E. 284, 332 Lioga, A. 330 Lioumbas, I. 164 Lipinska, A.P. 121, 147, 154, 229, 249 Lisec, J. 299 Liška, J. 297 Littman, S. 149 Liu, F. 144 Liulea, S. 206, 286, 350 Ljubešić, Z. 160, 273 Ljubimir, S. 288, 289 Llavero-Pasquina, M. 256 Lloyd, C. 162 Lo Martire, M. 287 Lobakova, E.S. 81, 172, 330 Lochmanová, G. 256 Lohr, M. 129 Lohse, D. 293 Loisel, S. 72

Lombardi, E. 100 Long, M. 160 Lopes dos Santos, A. 190, 227 Lopes Júnior, C.A. 191 Lopes, F. 322, 328 López-Adams, R. 133 Lopez-Bautista, J. 230 López-Rodríguez, M.C. 218 Lorenz, M. 175, 336 Lorenzo Vilizzi, L. 341 Loring, J. 339 Louvet, I. 54 Lucena Frédou, F. 291 Lučić, P. 219 Lukavský, J. 297 Lukicheva, S. 262 Lukyanov, A.A. 81, 172, 330 Lutz, S. 295

### Μ

Ma, N. 182 Macaya, E. 193 Mac-Bear, J. 121 Machado, C.B. 71 Macías, M. 192, 333 MacKechnie, K. 304, 311 Mackinder, L.C.M. 110, 125, 134, 255, 257, 258 Mackintosh, A. 179 Maddix, G.-M. 71 Madeira, P. 147 Mäder, P. 336 Madhuri, S. 111 Maes, A. 132 Maga, E. 161 Mager, S. 157 Maggs, C.A. 47, 51, 189 Magnabosco, C. 65 Maguer, J.F. 119 Mairet, F. 119, 240 Malea, L. 168

Malihan-Yap, L. 268 Malis Arad, S. 86 Malta, E.-j. 57, 333 Manat, G. 121 Manchado, M. 351 Mandić, J. 210 Maneveldt, G. 49 Manfellotto, F. 100, 157 Mangialajo, L. 115, 287 Manirakiza, E. 54, 234, 253 Mann, D.G. 143 Marani, G. 207 Marchal, L. 316 Marchand, J. 263 Marchand, L. 323 Marchetto, F. 269 Marchi, F. 147 Marec, G. 262 Maréchal, E. 105 Maree Smith, S. 282 Mareš, J. 66 Margaret, B. 61 Margiotta, F. 100 Maridakis, C. 220 Marie, A. 115 Marie, D. 190 Markov, G. 62, 313 Marks, S. 305 Marsh, R. 71 Marteinsson, V.T. 57 Martín, D. 282 Martin, T. 322, 328 Martínez Gutiérrez, C. 307 Martínez Yerena, J.A. 66 Martínez, B. 73, 227 Martini, F. 287 Martín-Martín R.P. 91, 248 Martins, B. 341 Martins, F.M.S. 97 Martins, G.M. 349 Martins, N. 64, 99, 147 Mary, L. 95

Mas, J.A. 337 Maskrey, B.H. 313 Massé, C. 72 Matard-Mann, M. 310 Matek, A. 160, 273 Matić, F. 225 Matoju, I. 93 Matsuda, Y. 104, 125, 126, 129 Matthijnssens, J. 243 Mauger, S. 123, 145, 147 Maumont, S. 338 Mazéas, L. 102, 231 Mbaye, M.S. 212 McCann, M. 99 McCarthy, J. 303 McCormick, A. 125 McHale, M. 126, 185, 303 McKenzie D. 173 Mear, H. 78 Medlin, L.K. 101 Meeda, Y. 109 Meermann, B. 191 Mehlmer, N. 261 Meier, S. 336 Meire, P. 264, 346 Meland, H. 179 Méléder, V. 58, 162, 271, 310 Melero-Jiménez, I.J. 285 Melo, R. 179 Memmola F. 93 Mendoza-Ochoa, G.I. 256 Meneghesso, C. 95 Menicot, S. 54 Menneteau, M. 176 Mente, M.S. 330 Meriot V. 270, 315 Meroni, L. 115 Mertens, A. 143 Meshulam, D. 86

Messyasz, B. 220, 251, 283, 338 Metegnier, G. 95 Mével, G. 176, 266 Meyer-Klaucke, W. 103 Michalak, I. 308 Michaloudi, E. 164 Michel, G. 238, 253 Michel, T. 79 Michelsen, S. 290 Michotey, V. 291 Miclon, M. 310 Middelburg, J.J. 159 Mieszkowska, N. 158 Migliore, G. 328 Migné, A. 72 Mihanović, H. 273 Mijovilovich, A. 103 Millar, J.L. 190 Miller, E.P. 103 Miller, M. 174 Mindiola, K. 56 Minio, M. 267 Minne, A. 276 Mir, Y. 239 Míšková, A. 133 Mistri, M. 223 Mittag, M. 259 Mock, T. 185 Mohamed Dahlan, M.F. 271 Mohamed, A.R. 149 Mojzeš, P. 109, 245 Mollica, T. 282 Mollo, L. 331 Monserrat, M. 115, 287 Monteiro, C. 95, 96, 97, 119 Montereali, F. 324 Monterio, J. 73 Montresor, M. 65, 100, 157 Moon, J.S. 329 Moore, P.J. 159, 265 Mora, T. 121

Morales-Pineda, M. 152 Morelle, J. 131, 346 Moreno-Garrido, I. 291 Morgado, D. 322 Moriceau, B. 119, 160, 272 Moro, I. 183, 223 Morozzo Della Rocca, B. 324 Morrison, H.G. 237 Moschandreou, K. 342 Moschin, E. 183, 223 Mossion, A. 58, 310 Mostovaya, A. 167 Motomura, T. 247 Mouget, J.L. 98 Mountourakis, F. 330 Mouritsen, L.T. 182 Mourot, R., 299 Moustaka, M. 164 Moye, J. 195 Mozetič, P. 164, 225 Mozgová, I. 256 Msuya, F.E. 93 Mucko, M. 273 Muguerza, N. 120 Mukherji, R. 259 Mulder, M.T. 181 Müller, D.G. 155 Müller, K.M. 233 Munari, C. 223 Munari, M. 287 Munaron, J-M. 291 Muñoz L. 60, 240 Murik, O. 153 Murison, V. 263 Murúa, P. 60, 186, 240 Musiał, S. 257

### Ν

Nadermann, J. 351 Nadir Solak, C. 288 Nagasato, C. 247

Nakayasu, M. 129 Nakou, K. 168, 287 Nam, O. 134 Nappi, J. 234, 235, 242 Narayanan, R.A. 332 Nardiello, M. 287 Nascimento, S.M. 285 Nasto, I. 285 Navalho J. 170 Navarro, N. 337 Navarro-Martínez, P.C. 337 Naveira, C. 285 Navon, M. 177 Navrozidou, V. 342 Ndawala, M. 93 Ndiaye, W.N. 203, 212, 277, 278 Necchi Jr., O. 205 Nedbalová, L. 48, 297, 299 Nef, C. 121, 144 Nehr, Z. 170, 249 Neiva, J. 72, 99, 222 Nejašmić, J. 219 Nelly, A. 305 Nelson, W.A. 50, 181 Nemcova, Y. 273 Nerantzis, E. 330 Neri, F. 93 Neu, A.-K. 294 Neuhauser, S. 186 Neustupa, J. 273 Neves, R.A.F. 285 Neveu, C. 310 Newsad, S.A. 256 Newville, M. 103 Nham, Q. 332 Niedzwiedz, S. 192, 298 Nielsen, M.M. 167 Nieto Vilela, R 96 Nigishi, M. 126 Nikkanen, L. 268 Nikolopoulou, I. 342

Nilsen, A.M.L. 179, 186 Nilsson, S. 307 Ninčević Gladan, Ž. 225 Nirma, C. 49 Nishihara, G.N. 274 Nitschke, M.R. 70 Noël, C. 56 Noisette, F. 276 Norici, A. 106, 200, 331 Normant, V. 258 Nöthig, E-M. 195 Nour. M. 323 Novak Vánclová, A.M.G. 144 Nowak, P. 183 Nunes, N. 323 Núñez, Á. 339 Núñez, T. 337 Nurdin, N. 76

### 0

O'Connell, C. 93 Oakley, C. 174 Ocshenkühn, M.A. 149 Ødegård, J. 146 Oetterer, A.P. 158 Oiry, S. 76, 166, 221 Oliveira, M. 147 Olsen, L. 179 Omand, M. 273 Orefice, I. 65 Orenes-Salazar, V. 337 Orfanidis, S. 168, 287, 330 Orlando-Bonaca, M. 273 Orlić, M. 273 Ortenzi, F. 324, 328 Oschatz, C. 108 **OSPAR ICG-EMO 166** Otero-Ferrer, F. 47 Otte, J. 138, 139

### Ρ

Pageault, L. 123, 240 Palatini, J. 269 Palma, G. 127, 193 Pan, L. 157 Pang, S.J. 85 Pankiewicz, R. 338 Papadimitriou, A. 168, 287, 330 Papazi, A. 330 Parages, M.L. 301 ParedesChi, A. 334 Parente, M.I. 224, 349 Pargana, A. 156 Park, H. 156, 241, 247 Park, J. 205 Park, Y.J. 318 Parkes, R. 81 Pascal, F. 93 Pascal, P-Y. 279, 342 Paterová, A. 299 Paterson, D.M. 344 Patiño, D. 60 Patron, L. 149 Paul, C. 113 Paulino-Le Deun, S. 56, 235, 241 Peace, A. 113 Pearson, G.A. 72, 99, 147 Pecquerie, L. 291 Pedersen, M.F. 159 Pedersen, S.G. 167 Pejković, M. 210, 212 Peltekis, A. 176, 266 Peltier, G. 125 Peña, V. 47, 206, 218 Pena-Martín, C. 337 Penna, A. 212 Peplow, L.M. 70 Pereira, H. 170 Pereira, J. 95, 96

Pereira, L.F. 118 Pereira, M. 234 Perez de Souza, L. 126 Pérez, E. 245 Perez, S. 238 Perez-Fernandez, M. 85 Pérez-Lloréns, J.L. 198 Pérez-Santos, I. 127 Permann, C. 105 Perrin, A.J. 255, 258, 337 Persillon, C. 85 Pessarrodona, A. 159 Peteiro, C. 306 Petek, S. 49, 241, 323 Petelin, B. 164 Peter, C. 284 Peter, E.K. 299 Peters, A.F. 154 Peters, R.S. 191, 299 Petersen, M. 138 Petrucciani, A. 106 Pezy, J.-P. 72 Pfannkuchen, D.M. 202, 215 Pfannkuchen, M. 202, 215 Philippi, M. 161 Pickford, R.E. 295 Pierella Karlusich, J.J. 187 Pierre-Elies, P. 141 Piganeau, G. 234 Pilátová, J. 109, 245 Piñeiro-Corbeira, C. 275 Pinheiro de Carvalho, M.Â.A. 323 Pintado, J. 59, 234, 235, 242, 265 Piredda, R. 217 Pirovano, W. 143 Pivato, M. 211 Piwosz, K. 133 Piwowarczyk, R. 203 Plag, N. 192 Plantier, V. 230

Plestino, E. 147 Pliego-Cortés, H. 77 Plus, M. 155, 166 Pochic, V. 90 Pochon, X. 174 Podolšak, I. 215 Poggiale, J-C. 291 Poidevin, A. 78 Poirier, L. 271 Pokorný, J. 42 Poletto Borges, V. 233 Polsenaere, P. 162 Polykarpou, P. 164 Pondaven, P. 90 Poong, S.W. 46 Popper, Z.A. 311, 319, 326 Poquita-Du, R.C. 139 Potin, A. 222 Potin, D. 146, 170, 249 Potin, P. 146, 170, 249, 313 Poyatos, F. 245 Pradelles R. 181 Prado, S. 236 Prášil, O. 133 Prelle, L.R. 122 Prestes, A. 349 Přibyl, P. 297, 299 Price, B. 208 Prigent, L. 155 Priouzeau, F. 115 Probert, I. 131, 190 Procházková, L. 48, 78 Pröfrock, D. 298 Pruvost, J. 316 Puente, A. 167, 222, 251 Puginier, C. 138 Pula, H.J. 334 Purbani, D.C. 98 Putnam, H. 294

### Q

Quack, B. 203, 212, 277, 278

Queiroga, F.R. 285 Queirós, A.M. 159 Quéré, J. 95, 155 Quesada-Calderón, S. 231 Quétel, I. 215 Quigley, C.T.C. 237 Qui-Minet, Z.N. 59, 242 Quintano, E. 120

### R

Rabiller, E. 116 Radini Noerdjito, D. 98 Rad-Menéndez, C. 304, 311 Raimbault, P. 237 Raimondi, P. 72 Ramanan, R. 187 Rami, M. 348 Ramos, E. 167 Rancel-Rodríguez, N.M. 206, 286 Rao, A.K. 58 Raoux, A. 72 Ratchinski, P. 154 Rautenberger, R. 179 Ravanel, S. 260 Ray, S. 306 Reddy, M.M. 99 Reed, K.B. 158 Rehder, L. 132 Reiss, H. 179 Reissig, L. 108 Reitzel, H. 281 Remias, D. 48, 78 Rengefors, K. 88 Repecaud, M. 177 Retif, J. 271 Reul, A. 285 Réveillon, D. 119 Reyes, A. 312 Reyes, J. 206, 286 Rhodes, L. 174 Ribeiro, C. 341

Ribeiro, L. 166 Ribera d'Alcalá, M. 110, 187 Richards, J. 201 Richardson, E.H. 144 Richier, S. 280, 292 Rickaby, R.E.M. 141 Riera, P. 60 Rieusset, L. 56, 79, 235 Rijsdijk, N. 250 Rindi, F. 183 Ríos Vázquez, A. 334 Rivière, P. 272 Rizzo, A. 271 Roberts, W.R. 143 Robinson, G. 93 Robledo, D. 207, 291 Rodolfo, C. 84 Rodrigues A. 170, 349 Rodrigues, N. 285 Rodríguez, F. 95 Rodríguez, J. 286 Rodríguez, S. 349 Rodríguez-Prieto, C. 206 Rogato, A. 110 Rogniaux, H. 121 Roje Busato, R. 212 Rokitta, S.D. 132, 195, 300 Romagnoli, T. 93 Román Muñoz, A. 73, 348 Romano, G. 65, 83 Romero Rodríguez, I. 116 Romero, G. 115, 287 Romero, J. 245, 335 Romero-Campero, F.J. 152, 244 Romero-Losada, A.B. 152 Ronchi, P. 247 Ropartz, D. 121, 238 Roret, T. 238 Rosa, P. 162, 221 Rosain, D. 165 Rosas-Guerrero, J. 339, 348

Rosignoli, S. 185 Ross, M.E. 85, 304, 311 Rossel, P.E. 299 Rossi, A.M. 115 Rost, B.C. 89, 132, 195, 300 Rothman, M. 162 Rousseau, C. 53, 313 Rousseau, F. 47, 51, 207, 220, 224 Rousselot, E. 230, 233 Rousvoal, S. 53 Roux, C. 338 Rowe Davies, B.F. 221 Rowell, J. 304, 311 Ruggiero, A. 157 Ruggiero, M.V. 100 Rugnini, L. 324, 328 Ruitton, S. 349 Ruiz Gonzalez, C. 93 Ruiz P. 59, 235, 242 Rull Lluch, J. 248 Rusekwa, S. 93 Russo, M.T. 110, 157 Ruttink, T. 146 Ryan, I. 303 Ryan, K. 174 Ryan, W.H. 158

### S

Sabbe, K. 56, 156, 262, 264, 346 Sadogurska, S.S. 223 Saenz-Aguledo, P. 231 Saha, M. 63 Saint-Jean, B. 240 Saint-Sorny, M. 85 Sainz-Villegas, S. 222, 251 Salazar, J. 325 Salgado Murillo, B. 162 Sallen Cruz, C. 213 Salvo, F. 91 San Gil, C. 206

Sánchez de Pedro, R. 128, 285, 301 Sánchez-Astráin, B. 222, 251 Sandoval, J. 312 Sanges, R. 157 Sangil, C. 44, 286 Sansón, M. 44, 206, 286 Santaeufemia, S. 269 Santamaría, J. 282, 287 Santin, A. 110 Santos, A. 349 Santos, F.S. 285 Santos, L.M.A. 305 Santos, R. 350 Sarno, D. 100, 217 Sasi A. 173 Sassi, J-F. 85 Sasso, S. 107 Sato, Y. 274 Saubrekka, K. 216, 301 Saucède, T. 51 Saunders, G.W. 45 Sauvage, T. 201 Savadova-Ratkus, K. 287 Savela, H. 283 Savio, S. 84, 324, 328 Savoie, A. 296 Saxon, R. 304, 311 Schäfer, S. 350 Scharfenstein, H.J. 70 Schaum, E. 89 Scherbakov, P.N. 81, 330 Scheschonk, L. 186 Schiffrine, N. 141 Schils, T. 49 Schmedes, P.S. 167 Schmid, M. 185 Schmider, A. 193 Schmidt, A. 92 Schmidt, C.E. 298 Schmidt, Wm. 201 Schmitt, I. 139

Schmitt, S. 116 Schneider, C.W. 45 Schnetzer, J. 161 Schoefs, B. 263 Schreiner, M. 203 Schubert, H. 183 Schubert, P. 226 Schwehm, N. 161 Schweibold, L. 90 Sciuto, K. 183, 223 Scornet, D. 147, 154 Scott, R. 337 Scuderi, F. 328 Seabloom, E.W. 113 Seabra, R. 95, 118 Seitidou, O. 168 Sellés, J. 187 Selyakh, I.O. 81 Semba Prasetiya, F. 98 Semenova, L.R. 81 Sepe, R.M. 65 Serais, O. 215 Serif, M. 111 Serôdio, J. 130, 131, 346 Serrão, E.A. 64, 72, 99, 147, 222, 237, 350 Serre-Fredj, L. 177 Serviere-Zaragora, E. 312 Sevin, L. 72 Sfriso, A. 223 Sfriso, A.A. 223 Shan, T.F. 91, 192 Shashibhushan, N.B. 332 Shibzukhova, K.A. 172 Shim, E. 156, 241, 247, 250 Shimakawa G. 104, 125, 126, 129 Siano, R. 95 Sibat, M. 119 Siegel, A. 62, 313 Silkina, A. 79 Silvestrini, C. 287

Sim, C.W.H. 190, 227 Simmler, C. 79 Simon, C. 126, 303 Simon, N. 55 Simon-Bouhet, B. 342 Sirin, S. 325 Sitjà, C. 282, 287 Sjøtun, K. 224 Skaloud, P. 138 Skejić, S. 225 Skjermo, J. 248 Skoupý, S. 43, 207 Slavinec, P. 164, 225 Slee, N.J.D. 135 Śliwińska, M.A. 269 Smale, D.A. 159, 265 Smerdou, C. 192, 297, 301 Smillie, I. 268 Smith, A.G. 121, 256 Smith, K. 174 Smith, S. 74, 276, 293 Smodlaka Tanković, M. 202, 215 Šnokhous, K. 297 Soler Onís, E. 343 Solovchenko, A.E. 81, 172, 330 Solymosi, D. 269 Song, D-G. 318 Sonko, A. 278 Sourisseau, M. 90, 95 Sousa Pinto, I. 159 Sousa, A. 221 Spinelli, S. 267 Sreekumar, S. 256 Stachowski-Haberkorn, S. 240 Stahl, F. 272 Stallforth, P. 259 Stamatis, N. 330 Stamou, G. 164 Stanley, M.S. 85, 311

Stanojković, A. 43, 207 Stearns, L.A. 237 Stefanidou, N. 164 Steinberg, R. 116, 226 Steinhagen, S. 54 Štenclová, L. 42, 66 Stengel, D.B. 319 Stewart, A. 98 Stiger-Pouvreau, V. 49, 83, 87, 214, 278, 291, 312, 317, 320, 323 Stosic, B. 291 Straka, M. 225 Strugała, M. 251 Stubbs, K. 238 Sturbois, A. 94 Šubrt, D. 336 Šuikaitė, I. 287 Suikkanen, S. 283 Sulpice, R. 126, 185, 249, 303 Supraha, L. 281, 301 Suzuki, N. 159 Sveinsdóttir, H.I. 314 Sydorenko, A. 339 Szymański, J. 269

### Т

Tamburello, L. 287 Tamsouri, N. 348 Tan, J. 46 Tanaka, A. 54, 105, 274 Tang, J. 43 Tang, K. 79 Tanguy, G. 53, 60 Taupin, L. 56 Taylor, D. 167 Tebbani, S. 322 Ten-Hage, L. 338 Terradas, M. 337 Terrapon, N. 102, 231 Terrin, S. 280

Thibaut, T. 73, 87, 123, 207, 276, 291, 349 Thiébaut, E. 72 Thielecke, A. 161 Thierry, A. 337 Thomas, F. 60, 238, 242 Thomas, H. 298 Thomas, N. 304, 311 Thomas, O.P. 99 Thomas, S.-L. 71 Thomas, T. 235, 242 Thompson, L. 174 Thomsen, M. 182 Thornton, B.M. 158 Thouard, E. 87, 278 Tienken, D. 149 Timmermann, K. 167 Tirichine, L. 54, 67, 143, 230, 233, 234, 248, 253, 259 Tolu, J. 114 Tomašević, T. 210, 212 Tompkins, E. 197 Tonon, T. 71 Torres, R. 127 Totti, C. 93 Touboul, D. 49 Toumi, C. 94 Touzet, N. 81 Tran, J. 294 Tranter, M. 295, 299 Trautmann, M. 192 Trefault, N. 63 Treillefort, M. 91 Trentin, R. 183 Tretiak, S. 319, 326 Triay-Portella, R. 73, 227, 350, 351 Triba, M.N. 115 Tricoche, M. 294 Trobajo, R. 143 Tronholm, A. 44 Trottier, C. 54

Truong, T.Q. 318 Tsiaoussi, V. 342 Tsirliganis, N.C. 330 Turnbull, G.A. 173 Tuya, F. 73, 226

### U

Ubaldi, M. 93 Uhlig, S. 281 Ujević, I. 212 Uji, T. 247 Ulmann, L. 263 Underwood, G.J.C. 56, 135, 344 Urbschat, N. 300 Uribe, G. 290 Uroz, S. 60 Usache, V. 181 Utge, J. 145 Uthanumallian, K. 140 Uwizeye, C. 105

### V

Vader, A. 301 Valdazo, J. 351 Valero, M. 91, 99, 123, 145, 146, 147 Vallet, M. 117 Valskys, V. 338 Valverde, M. 337 van Bodegom, P. 143 van de Vijver, B. 143 van de Waal, D.B. 113, 281 van den Wyngaert, S. 113 van der Loos, L.M. 54, 243 van der Plank, S. 197 van der Wal, D. 264, 346 van Eijk, I. 143 van Oppen, M.J.H. 70 van Tussenbroek, B. 161 van West, P. 240 Váncl, A. 144

Vandepoele, K. 156, 250, 262 Vardi, A. 150 Varela, J. 170 Vascotto, I. 164 Vasilieva, S.G. 81, 172, 330 Vásquez-Elizondo, R.M. 291 Vaulot, D. 175, 190, 227 Vázquez Delfín, E. 334 Veillet, F. 62 Veluchamy, A. 253 Venera-Ponton, D. 201 Verbruggen, H. 50, 140 Verdu, P. 145 Verdura, J. 115, 287 Vergara, J.J. 198 Vergés, A. 282 Verlaque, M. 276 Viard, F. 72 Vigor, C. 181 Villegas, K. 127 Vinbæk, S.J. 167 Vincens, P. 229 Vincent, F. 150 Viruel, M. 285 Vis, M.L. 197, 205 Vitale, L. 100 Vitulo, N. 211 Vlašiček, I. 202, 215 Vonshak, A. 314 Vuong, T. 259 Vyverman, W. 56, 156, 250, 262

### W

Waeles, M. 214, 323 Wagner, A. 123 Wahbi-Fusier, K. 345 Wäldchen, J. 336 Walde, M. 55, 160 Walker, C.E. 125 Wallace, M. 238

Walusiak, E. 338 Wang, L.Z. 85 Watson, R.J. 304 Wawrzynkowski, P. 120 Webber, M. 71 Weinberger, F. 63, 212, 275, 278, 294 Wen, X. 244 Wernberg, T. 72, 159, 178 Werner, E. 195 Wetherbee, R. 140 Wey, L.T. 268 Wheeler, G. 108, 109 White, L. 162 White, O. 208 Wichard, T. 59, 242 Wiebe, C. 183 Więckowski, M.R. 269 Wielgosz-Collin, G. 58, 310 Wiese, J. 63 Wieters, E. 162, 213 Wigger, K. 179 Wilbraham, J. 184, 208 Wilhelm, C. 111 Wilk-Woźniak, E. 338 Willems, A. 54, 243 Williams, T.A. 140, 190 Williamson, C.J. 140, 190, 295 Willis, A. 68, 302, 304 Wilmotte, A. 174 Wilson, W. 61 Wiltshire, K. 226 Wincker, P. 69 Winkel, L.H.E. 114 Wishkerman, A. 326 Witt, J.D.S. 233 Wiwin, N. 98 Wojciechowska, R. 203 Wolf, K.E.K. 89, 100, 300 Wolf, M.A. 223 Won, B.Y. 201

Wood, S. 174 Wörtwein, T. 229 Wu, T-D. 60 Wu, Y. 67, 234, 253 Wulff, A. 307 Wynn, O.J. 263 Wysor, B. 201

### X

Xanthopoulou, N. 164 Xiao, S. 138

### Y

Yamada, N. 135 Yamagishi, K. 126 Yang, F. 234, 248 Yang, Y. 298 Yang, Z. 138 Yangrae Rho, Y. 205 Yashiro, E. 104 Ye, Z. 316 Yebra, A. 334, 335 Yee, D. 58, 61 Yesson, C. 184 Yoneda, K. 129 Young, J. 141

### Ζ

Zakka, E.E. 153, 252 Zalouk-Vergnoux, A. 271 Zammit, G. 209 Zanolla, M. 339, 348 Zarif, M. 259 Zaytsev, P.A. 81 Zdráhal, Z. 256 Zervas, A. 295 Zhang, D. 256 Zhang, M. 162 Zhang, Q.Y. 93 Zhang, R. 298 Zhao, G.Z. 255, 258 Zhao, Y. 327 Zheng, M. 155, 247 Zhong, B. 138 Zonnequin, M. 62 Zubia, M. 294, 308 Zuccarello, G.C. 156 Žuljević, A. 219