



Keynote and Oral Papers

To cite this article: (2019) Keynote and Oral Papers, European Journal of Phycology, 54:sup1, 31-117, DOI: [10.1080/09670262.2019.1626627](https://doi.org/10.1080/09670262.2019.1626627)

To link to this article: <https://doi.org/10.1080/09670262.2019.1626627>



Published online: 20 Aug 2019.



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Keynote and Oral Papers

1. Phylogeny in the ‘omics’ era: new approaches for the study of the evolution of algae and their organelles

1KN.1

DIVERSIFICATION TRENDS IN THE ARCHAEPASTIDA INFERRED FROM CHLOROPLAST GENOME DATA

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Thanks to high-throughput sequencing, it has become easier and cheaper than ever to generate genome-scale data for a broad array of non-model species. This offers great perspectives to improve our understanding of algal classification and evolutionary biology. We inferred phylogeny of the Archaeplastida using >460 chloroplast genomes and calibrated in time using 19 fossil occurrences, providing a mostly resolved picture of the phylogenetic history of the group. We used the time-calibrated phylogeny to investigate patterns of diversification through time and across lineages, showing an increase of lineage accumulation during the Paleozoic, with stark differences between the contributions of different functional form-groups to overall diversification.

1KN.2

AUTOMATED BIOINFORMATICS APPROACHES TO EVALUATE THE RELATIVE CONTRIBUTION OF ENDOSYMBIOSIS AND KLEPTOPLASTY IN THE EVOLUTION OF COMPLEX RED ALGAE

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Complex algae form a network of photosynthetic organisms spread across the eukaryotic tree. Among

those, CASH lineages have plastids related to red algae, but the mechanisms by which they were acquired remain unclear. Contrary to expectations of secondary endosymbiotic models, a sizable part of their plastid-targeted genes is not from the same origin as the plastid itself. Scenarios as the *ancestral cryptic serial endosymbioses* or the *shopping bag model* provide a substantial interpretation of this gene mosaicism but are exclusively focusing on an endosymbiotic mechanism. Similarly, while the *Rhodoplex* hypothesis fits the single phylogenetic origin of complex red plastids, it does not explain gene mosaicism. Moreover, it implies multiple tertiary (or quaternary) endosymbiotic events, whereas most complex algae do not bear nucleomorphs and only have 3 or 4 membranes surrounding their plastids. To overcome the inconsistencies of those models, we propose kleptoplasty as an additional mechanism for explaining plastid spread in CASH lineages. In line with the *shopping bag model*, our hypothesis posits multiple transient interactions with preys of diverse origins but also proposes a rationale for the selective force driving the progressive accumulation of plastid-targeted genes: to maintain functional kleptoplastids for increasingly longer periods of time, before ultimately reducing them into fully integrated plastids. In such a scenario, the phylogenetic diversity of plastid-targeted genes would be higher than predicted with endosymbiotic models, where genes originate mostly from a single source, the algal symbiont. To test our hypothesis, we designed and implemented an automated pipeline for building and parsing gene trees in a discovery-driven and taxonomy-aware fashion. This approach allows us to infer whether any given CASH alga rather arose through endosymbiosis or kleptoplasty by reading its position on an alpha-diversity ladder, ranging from (theoretically) single-source nucleomorph-bearing organisms (e.g., *Guillardia theta*) to multiple-source kleptoplastic organisms (e.g., *Dinophysis acuminata*).

1OR.1**FACILITATED MITOCHONDRIAL EVOLUTION OF AN EXTREMOPHILIC RED ALGAE**

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It is generally fatal in harsh conditions to the most of species, but some species can thrive in extreme environments including high temperature, acidic, and heavy-metal rich conditions. Compare to many prokaryotic extremophiles, only a few eukaryotic species are reported from this environment. The Cyanidiophyceae (Rhodophyta) are the best eukaryotic example that thrive in acidic (pH 0-2), and high-temperature habitats (35-50°C). Study on this interesting organism, however, is poorly conducted. To fill the gap of our understanding of the cyanidiophycean biology, we have completely sequenced and annotated five new mitogenomes from the representative clades of Cyanidiophyceae. In this study, we addressed two different issues; i) resolving the phylogenetic relationship and ii) the classification of mitochondria type in Cyanidiophyceae. To this end, we compared trees based on a concatenated gene set and sub-sampled datasets. The relationship between the major lineages of Cyanidiophyceae has been resolved from our analysis. We also distinguished two mitochondrial types, *Cyanidium*-type and *Galdieria*-type, based on their distinctive features of mitogenomes. The result represents that a strong selective pressure affected mitochondria of *Galdieria*-type, which are altered the protein properties of mitochondrial genes. Based on comprehensive genomic analyses, we will provide insights into the evolutionary history of Cyanidiophyceae.

1OR.2**PHYLOGENOMICS OF THE RED ALGAL ORDER CERAMIALES: IMPLICATIONS FOR FAMILY LEVEL CLASSIFICATION**

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The red algal order Ceramiales was previously arranged on the basis of morphological studies into five families: Ceramiaceae, Dasyaceae, Delesseriaceae, Rhodomelaceae and Sarcomeniaceae. The first phylogenies based on one or two molecular markers failed to produce well-resolved phylogenies or to resolve the first three families as monophyletic. At present, nine families are recognized in the Ceramiales after the split of the Ceramiaceae into five families. Moreover, the Delesseriaceae and Dasyaceae include three and two subfamilies, respectively. However, relationships among families and subfamilies are poorly understood. Recent advances in High Throughput Sequencing techniques facilitate the sequencing of plastid genomes, which have been used for resolving challenging phylogenies in the red algae. In this study, we attempted to produce a well-resolved phylogeny for the order Ceramiales using 80 (27 newly sequenced) plastid genomes representative of the major lineages. We aligned and concatenated 208 plastid coding sequences (CDSs) that we used to construct a maximum likelihood tree. The resulting phylogeny resolved most of the nodes with full support and showed that a family-level reclassification is required. The Rhodomelaceae, Ceramiaceae and Wrangeliaceae were resolved as monophyletic and we propose to maintain their current circumscription. By contrast, the families Callithamniaceae, Dasyaceae, Delesseriaceae, Sarcomeniaceae, Spyridiaceae and Inkyuleaceae require revision.

1OR.3**“RAPPEMONADS” IS THE THIRD LINEAGE OF HAPTOPHYTA**

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“Rappemonads” is a monophyletic plastid-bearing lineage that is comprised of only environmental DNA sequences in the plastid 16S rRNA tree. “Rappemonads” is not nested in any of algal clades in the phylogenetic tree, and therefore “Rappemonads” is thought to be a novel lineage independent from any of known major algal groups. However, because no one could have succeeded in the observation of living cells of “Rappemonads,” identity of this eukaryotic

group has been unknown. In this study, we found “Rappemonads” as a novel haptophyte lineage. The haptophyte strain NIES-3900 was isolated in and had been maintained from 1990. Cells of the haptophyte strain NIES-3900 have a haptonema between two flagella, and four or more plastids. HPLC analysis unveiled that the haptophyte strain NIES-3900 possessed two unidentified pigments in addition to pigments known to be detectable in other haptophyte species, such as chlorophyll *a*, chlorophyll *c2*, chlorophyll *c3*, and fucoxanthin. The number of plastids and the pigment composition indicate that NIES-3900 is distinguishable from the two classes of haptophytes, Prymnesiophyceae and Pavlovophyceae, and thus this haptophyte strain is to be recognized as a novel species belonging to a novel, third class of haptophytes. Phylogenetic analyses of 18S rRNA gene sequences, mitochondrial genomes, and plastid genomes strongly suggest that the haptophyte strain NIES-3900 is the sister lineage of Prymnesiophyceae and that Pavlovophyceae is the deepest lineage of haptophytes. To explore environmental DNA sequences that correspond to the lineage to which NIES-3900 belong, we also performed phylogenetic analyses of plastid 16S rRNA gene sequences and environmental DNA sequences. Most importantly, NIES-3900 was reconstructed as monophyletic with environmental sequences previously identified as “Rappemonads.” Given these findings, we conclude here that “Rappemonads” is the third class of haptophytes.

1OR.4

COMPARATIVE PLASTID GENOMICS OF NON-PHOTOSYNTHETIC CHRYSOPHYTES: GENOME REDUCTION AND COMPACTION

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Many non-photosynthetic chrysophytes lost their red-algal derived plastid and evolved several times through independent parallel evolution within the Chrysophyceae. The *Spumella*-like flagellates were characterized by a naked cell surface, non-colonial, and heterokont flagella. Recently, the genera *Pedospumella* and *Poteriospumella* were erected as an independent lineage from *Spumella*-like flagellates based on molecular phylogeny. Although the member of *Spumella*-like flagellates lost their photosynthetic ability, they still possess a leucoplast and retain a plastid genome. Here, we report a comparative analysis of plastid genomes sequenced from the non-photosynthetic chrysophyceae: *Spumella*, *Pedospumella* and *Poteriospumella*. We carried out a detailed analysis of their genome structures and coding capacities relative to a published photosynthetic chrysophycean plastid genome sequences of *Ochromonas* species CCMP1393 and synurophycean algae. The plastid genome of *Spumella*-like flagellates is strongly related with other photosynthetic chrysophycean lineages in genome structure and gene contents. Not surprisingly, most of the gene loss was related with photosynthesis and carbon fixation, except *petF* encoding Ferredoxin. The three non-photosynthetic chrysophycean plastid genomes are highly conserved with respect to genome structure and coding capacity, with the exception of gene rearrangements and partial duplications at the boundary of the inverted repeat and single-copy regions. The plastid genome of *Spumella*-like flagellates ranged from ~55.7 Kbp (*Spumella* species) to ~62.9 Kbp (*Poteriospumella* species) and shared a core set of 60 functional protein-coding genes, 3 rRNAs, and 32~34 tRNAs. Several lineage-specific gene loss/gain events were detected. We also uncovered a few examples of gene loss/gain, duplication and gene rearrangement in an inverted repeat region. Our results provide new insights into the evolutionary history of organelle genomes via genome reduction and gene loss related to photosynthesis in chrysophycean evolution.

2. Ecology and evolution of algal symbiosis

2KN.1

FROM WATER TO LAND: EVOLUTION OF THE ARBUSCULAR MYCORRHIZAL SYMBIOSIS

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The plant lineage faced two major transitions over the last 450 million years: the colonization of land and the transition from a gametophyte- to a sporophyte-dominant lifestyle, resulting in the divergence of vascular plants. These two events required the evolution of new mechanisms and the recruitment of existing pathways in a new developmental context. The fossil record and its broad host range suggest that the Arbuscular Mycorrhizal symbiosis evolved in first land plants and was one of the critical innovations that allowed plants to successfully colonize lands. Studying the evolution of AM symbiosis in land plants thus offers a unique opportunity to understand how key innovations evolve and are recruited during plant evolution. We use combinations of phylogenomics, genetics in multiple land plants and biochemistry to understand how this complex trait evolved. Using a comprehensive phylogenetic analysis on more than 200 transcriptomes and newly sequenced genomes of land plants and green algae, we identified the evolutionary path leading to the emergence of symbiotic gene networks in land plants and we have validated the importance of these pathways in vascular and non-vascular plants. We predict that AM symbiosis evolved in land plants by the recruitment of pre-existing pathways, neo-functionalization of recently diverged paralogs and gene fusion. The development of algal models will in the future allow us to further test this prediction.

2OR.1

A 'MISSING LINK' DINOFLAGELLATE *DURINSKIA KWAZULUNATALENSIS* EXHIBITS THE CONVERSION PROCESS OF THE KLEPTOPLASTIDS TO PERMANENT ENDOSYMBIONTS

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'Dinotoms' are dinoflagellates possessing endosymbiotic diatoms (ESDs) as tertiary plastids. The ESDs are evolutionary intermediate plastids: they still retain nuclei, mitochondria, ribosomes, ER, as well as the plastids, and the ESD nuclei remain mostly functionally intact. However, although the ESDs in dinotoms are genetically and structurally independent from their host cells, it has been reported that the host dinoflagellates maintain their ESDs permanently by controlling the ESD karyokinesis. Recently, we found that the ESDs of the dinotom *Durinskia capensis* and its close relative *D. kwazulunatalensis* show atypical evolutionary stages when compared to other dinotoms. The former species is a kleptoplastic dinotom, which can keep the ESD only for two months, due to the inability of the ESD nucleus replication. *D. kwazulunatalensis*, on the other hand, can maintain the ESDs permanently similarly to other dinotoms, while possessing three to six multiple ESD

nuclei in the single cells. Because all other dinotoms possess an ESD nucleus in each cell, and the *D. capensis* is still in kleptoplastic stage, we suggest the ESDs of *D. kwazulunatalensis* have evolved only recently from kleptoplastids to permanent endosymbionts. Interestingly, the ESD nuclei of *D. kwazulunatalensis* show unique morphological dynamics: the ESD nuclei change their morphology from round to string shaped, then disperse alongside the plastids during the host interphase, while changing back to a round shape to replicate, prior to host cell division. We further noticed that such a morphological change has been observed in permanent ESD nuclei of two other dinotoms, while never in any other eukaryotic nuclei, including the ESD of *D. capensis*. It is possible that the establishment of this novel nuclear dynamics might be a critical step for converting kleptoplastids to permanent endosymbionts.

2OR.2

MICROBIAL COMMUNITIES ASSOCIATED TO AN INVASIVE SEAWEED AND HOW THEY VARY ACROSS ECOLOGICAL SCALES

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Over the course of the invasion process, the red alga *Agarophyton vermiculophyllum* (synonym: *Gracilaria vermiculophylla*) has developed tolerance to several stressors, including epiphytic overgrowth. Therefore, the interaction between the seaweed host and the epiphytic community might have contributed importantly to the invasion success. However, little is known about the composition of these communities and how they vary across the wide distribution range of this invasive species. We collected *A. vermiculophyllum* from 6 populations in the native range (Japan, China) and 8 populations in the non-native range

(Germany, France, Virginia and California) and conducted high-throughput sequencing of partial 16S and 18S rDNA to characterize pro- and eukaryotic communities. Our data show that both the algal surface (epiphytes) and tissue (endophytes) host a set of 'core taxa' that are continuously associated across all collection sites. Aside this conserved subset of microbes, we found that communities are differently structured between the algal surface and tissue. Further, there was a strong spatial pattern, where individuals within the same population harboured similar communities whereas those were more different between populations. Our results indicate that the microbiome of *A. vermiculophyllum* contains a set of core taxa, which has been maintained across the native and non-native ranges. At the same time, site-specific differences across the ranges exist which require further investigation, as they could reflect adaptations during the invasion process.

2OR.3

PHOTOSYNTHETIC C & N UPTAKE OF SYMBIOTIC RADIOLARIA ASSESSED BY STABLE ISOTOPE ANALYSIS

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Acantharia and Collodaria, (Radiolaria, Rhizaria), are heterotrophic protists that can be found in the planktic realm across all oceans. All Collodaria and a large fraction of Acantharia live in symbiosis with microalgae belonging to the dinoflagellates and haptophytes, respectively, therefore exhibiting a mixotrophic behaviour. These radiolarians are abundant and ubiquitous in surface waters of oligotrophic oceans where they could account for up to 4% of the total primary production and as much as 20% of the surface production. Despite the key ecological roles of these protists in energy transfer, carbon flux and the biological carbon pump, physiological research focusing on this photosymbiosis has been lagging behind in comparison to our understanding of the similar relationship of benthic invertebrates, like corals. Likely, because of their delicateness and that the host-symbiont complex cannot be maintained in culture, the biochemical link and nutrient fluxes between symbionts and their hosts remains largely unexplored. In this study, we performed bulk

Carbon and Nitrogen stable isotope enrichment experiments, showing the uptake of carbon and nitrogen by symbiont photosynthesis for both Collodaria and Acantharia. Though nitrogen measurements for Acantharia were impeded by sample quantity, we can show the first measurements of carbon content for the Acantharian holobiont. Furthermore, we see a variation of photosynthetic carbon uptake depending on the

nitrogen source. Both Radiolaria seem to be better adapted in the uptake of ammonium, which in turn stimulates the carbon assimilation. Along with follow up bulk CN analysis in order to shed light on CN uptake rates and content for these Radiolaria, this study is a critical step to understanding and ultimately modeling of planktic photosymbiosis relationships and their roles in oceanic ecosystems functioning.

3. Biodiversity and biogeography of algae

3KN.1

UNFOLDING THE GLOBAL BIODIVERSITY PATTERNS AND STRUCTURE OF MARINE PLANKTONIC DIATOM COMMUNITIES IN THE WORLD OCEAN

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Plankton forms the base of the marine food web and plays an essential role in the global climate and biogeochemical cycles. The vast majority of ocean biomass and biodiversity is composed of these tiny creatures. Despite their global importance, very little is known about these biological entities. The advent of high-throughput omics approaches has revolutionized molecular biodiversity studies facilitating the understanding of biogeography, community assembly and ecological processes. Diatoms are one of the most diverse and ecologically prominent groups of phytoplankton. Although widely studied, their diversity and biogeographic distribution patterns are not well known. The metabarcoding data set generated from the biological samples collected during the *Tara Oceans* (2009-2013) global circumnavigation was used to elucidate diatom global biodiversity patterns and structure across the world oceans. Notably, this community-level molecular survey reveals a new estimate of the total number of planktonic diatom species, a considerable unknown and exceptionally high diversity in the Open Ocean, and complex diversity patterns across oceanic provinces. This work provides a comprehensive perspective on diatom distribution and diversity in the world oceans and elaborates interconnections between associated theories and underlying drivers. It demonstrates how metabarcoding approaches can provide a framework to investigate environmental diversity at a global scale, which is deemed as an essential step in answering various ecological research questions. Consequently, this approach also provides a reference point to explore how microbial communities will respond to environmental conditions.

3KN.2

PATTERNS AND DRIVERS OF SEAWEED BIODIVERSITY: SPECIATION AND DISPERSAL OF THE RED ALGAL GENUS *PORTIERIA* AND BROWN ALGAL ORDER *DICTYOTALES*

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Understanding the biogeographical processes underlying current biodiversity patterns is a central goal in ecology and evolutionary biology. We gain important insights in the evolutionary processes generating seaweed biodiversity patterns in two widespread clades, the red algal genus *Portieria* and the brown algal order Dictyotales, by applying DNA-based species delimitation methods, phylogenetic analyses, and probabilistic modeling of geographic range evolution. A first striking outcome of was that species diversity in the two clades is far greater than assumed based on formally described taxa. Although cryptic diversity is no exception in the marine environment, the degree of cryptic diversity found in *Portieria* and some genera of Dictyotales (e.g. *Lobophora*) is remarkable. Although a wide variation in species ranges was found, many species have restricted distributions, indicating overall low dispersal capacity. A clear pattern of high diversity was found in the tropical central Indo-Pacific, with declining diversity when moving away longitudinally as well as latitudinally from this region. Time-calibrated phylogenetic analyses indicate that the two clades are ancient (late Cretaceous to middle Jurassic) and likely originated in the region that is now the central Indo-Pacific. We find general support for a higher diversification rate in tropical regions, in particular, the central Indo-Pacific, and repeated dispersal events to peripheral regions. In the Dictyotales, two large clades, *Dictyota* and *Lobophora*, experienced a major burst of diversification in the central Indo-Pacific in the Paleocene, followed by dispersal outward. While

Lobophora diversified strictly in tropical regions, *Dictyota* also expanded towards temperate regions. Our analyses show that given the old age of the clades involved, and the large diversity of organisms with different traits, different mechanisms produced biogeographical patterns in seaweeds. Finally, our study shows once more that accurate delimitation of species is important for the understanding of distributions and diversification of tropical seaweeds.

3OR.1

GLOBAL COLONISATION AND DIVERSIFICATION IN THE TERRESTRIAL DIATOM SPECIES COMPLEX *PINNULARIA BOREALIS*

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Due to their immense diversity, the systematics of diatoms is complex and challenging. In particular, the biogeography and ecology of (pseudo)cryptic diatom species are highly understudied and there are only a few data available on species limits, diversity and the timing of lineage splitting within species complexes. To address these issues, we used the terrestrial diatom species complex *Pinnularia borealis* as a case study, employing phylogenetic analysis and environmental metabarcoding to study its diversity and biogeography. Sampling on multiple continents, including Antarctica, allowed for the development of an extensive reference database, comprising over 800 cultures. Phylogenetic analyses of the cultured material revealed an unprecedented high molecular diversity implying the existence of hundreds of previously undetected (pseudo)cryptic, presumably species-level, lineages which started diversifying between 25 and 36 Ma. Although several lineages seem restricted in their geographic distributions, others are present on multiple continents in both northern and southern hemisphere. Intra-lineage phylogeographic patterns were present in at least one widely-distributed lineage, and were estimated to have originated in the late Pleistocene. Although several lineages within the complex have the ability to disperse over vast

geographic distances, effective dispersal has not been sufficiently frequent to prevent speciation, resulting in the accumulation of numerous genetically distinct lineages with restricted geographical distributions. The importance of dispersal limitation and historical factors in shaping *P. borealis* communities was further confirmed by diversity differences between the Polar Regions: whereas lineage-level diversity of *P. borealis* is higher in the Arctic, the degree of intra-specific differentiation is higher in Antarctica. Altogether, our findings highlight that even relatively mobile micro-organisms can be subject to extreme lineage-diversification and that biogeographical patterns may vary widely even among closely related taxa.

3OR.2

METABARCODING DIVERSITY AND SEASONALITY IN THE MARINE PLANKTONIC DIATOM FAMILY CHAETOCEROTACEAE

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Environmental DNA metabarcoding constitutes a powerful approach to assess planktonic diatom diversity. We examined the performance of 18S rDNA V4-metabarcoding in revealing the species diversity in the diatom family Chaetocerotaceae at the LTER-MC station in the Gulf of Naples. To this aim, we analyzed chaetocerotacean metabarcodes obtained from plankton samples collected on 48 dates over three years. Phylogenetic results resolved the metabarcodes into solitary haplotypes, each comprising a modest number of metabarcodes, and terminal polytomies composed of multiple haplotypes, each with usually one haplotype containing the bulk of the metabarcodes. Solitary haplotypes and polytomies were considered molecular operational taxonomic units (MOTUs). A total of 53 out of the 73 MOTUs included a reference, usually matching the solitary or dominant haplotype, and could thus be identified. Distribution of the metabarcodes over the sampling dates revealed clear seasonal patterning. Species in cryptic species complexes generally were found in different or offset periods. A comparison with contextual LM data showed that the species commonly recognized in the LM data were also present in the contemporary metabarcode data.

Comparison of the phylogenetic results with those of commonly applied clustering revealed the following: the number of MOTUs recovered using clustering depended on the similarity cut-off threshold. Set the clustering threshold too close to 100% and numerous fringe haplotypes within phylogenetically delineated MOTUs dropped over it into “rare MOTUs.” Set it at a lower % and phylogenetically closely related MOTUs collapsed into single MOTUs. No magic cut-off exists for clustering at which results are anywhere comparable with those of phylogenetic assignment.

3OR.3

STABILIZING THE SEA LETTUCES: DNA SEQUENCES FROM TYPE SPECIMENS OF *ULVA* (CHLOROPHYTA) ILLUMINATE NOMENCLATURE AND BIOGEOGRAPHY

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The type species of *Ulva* is the well-known sea lettuce *Ulva lactuca* Linnaeus, but the genus also includes the tubular *Enteromorpha* gut weeds. Morphological development in the genus is so plastic and variable that species identification now relies largely on DNA sequences such as ITS, *rbcL* and *tufA*. Nomenclature nevertheless requires the correct application of typified names. To answer longstanding systematic questions in *Ulva* we analyzed the DNA of many type specimens. Partial *rbcL* sequences obtained from the Linnaean type of *U. lactuca* showed that it was not the most common northern European sea lettuce, as had been assumed for over 200 years, but instead was identifiable as the warm-water species known since 1813 as *Ulva fasciata* Delile (type locality: Alexandria, Egypt). Kützing's *Phycoseris lobata* 1847 from Chile (= *U. lobata* (Kützing) Harvey) also is the same species. DNA sequences, obtained by Sanger sequencing and more recently using NGS, showed that the holotype of *U. fenestrata* Postels & Ruprecht 1840

from Kamchatka and the lectotype of *U. stipitata* Areschoug 1850 from the Swedish west coast are conspecific. Thus, *U. fenestrata* is the correct name for the common boreal species previously known in Europe as *U. lactuca*. We hypothesize that Linnaeus' *U. lactuca* type was collected in the Indo-Pacific by one of his 'apostles'. *rbcL* sequences obtained from the type material of *U. pertusa* Kjellman 1897 from Japan are nearly identical to the types of *U. australis* and *U. laetevirens*, both described by Areschoug in 1854 from South Australia. The data indicate that these three names all belong to the same taxon. DNA sequences from historic specimens have allowed us to look back in time at distributions of *Ulva* species prior to modern global introductions, and address historic, taxonomic and biogeographic questions.

3OR.4

SPECIATION IN THE ENDEMIC LAKE BAIKAL CLADOPHORALES SPECIES FLOCK

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Ancient Lake Baikal in eastern Russia is a known center of freshwater diversity and many radiations. Lake Baikal also contains several endemic Cladophorales species and genera. While there is a great morphological range in the endemic Cladophorales (15 taxa, in 4 genera), recent studies have shown that all these taxa represent a monophyletic group nested within the genetically variable, but morphologically simple, genus *Rhizoclonium*. The Baikal clade shows remarkably low genetic variation (2.8% in the ITS rDNA marker), considering its wide morphological variation, indicating both recent radiation but also making species delimitation problematic. We wanted to determine if these morphological taxa were distinct species and possibly a case of *in situ* speciation. Eleven microsatellites, plus different genetic clustering methods, were used to address these questions. Microsatellite amplification revealed that most loci had more than two alleles, suggesting polyploidy, and needing different coding approaches. Two genetic clustering methods showed that some morphotaxa (e.g., *Chaetomorpha baicalensis*, *Chaetomorpha moniliformis*, *Cladophora compacta*) were clearly distinct and suggest reproductive isolation of these species. Other taxa were probably conspecific or were ambiguously assigned, confirming some taxonomic hypotheses obtained with rDNA. Our data shows that speciation

has probably occurred sympatrically in Lake Baikal, possibly through the mechanism of polyploidization. Further studies should address the mechanisms of polyploidization and its effects on the life cycle of these species and whether it has led to the morphology diversity of these algae.

3OR.5

SPECIATION IN PROTISTS: SPATIAL AND ECOLOGICAL DIVERGENCE PROCESSES CAUSE RAPID SPECIES DIVERSIFICATION IN A FRESHWATER CHRYSOPHYTE

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Though eukaryotic microorganisms are extremely numerous, diverse, and essential to global ecosystem functioning, they are largely understudied by evolutionary biologists compared to multicellular macroscopic organisms. In particular, very little is known about the speciation mechanisms which may give rise to the diversity of microscopic eukaryotes. It was postulated that enormous population sizes and ubiquitous distribution of these organisms could lead to a lack of population differentiation and therefore very low speciation rates. However, such assumptions have traditionally been based on morphospecies which may not accurately reflect the true diversity, missing cryptic taxa. In this study, we aim to articulate the major diversification mechanisms leading to the contemporary molecular diversity by employing a colonial freshwater flagellate, *Synura sphagnicola*, as an example. Phylogenetic analysis of five sequenced loci showed that *S. sphagnicola* differentiated into two morphologically distinct lineages approximately 15.4 Mya, which further diverged into several evolutionarily recent haplotypes during the late Pleistocene. The most recent haplotypes are ecologically and biogeographically much more differentiated than the old lineages, presumably because of their persistent differentiation after the allopatric speciation events. Our study shows that in microbial eukaryotes, species diversification by colonizing new geographic regions or ecological resources occurs much more readily than was previously thought. Consequently, divergence times of microorganisms may be in some

lineages equivalent to the estimated times of speciation in plants and animals.

3OR.6

EVOLUTIONARY HISTORY OF CORALLINE RED ALGAE (CORALLINOPHYCIDAE, RHODOPHYTA) INFERRED FROM A MULTILOCUS TIME-CALIBRATED PHYLOGENY

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The subclass Corallinophycidae encompasses four orders of benthic red seaweeds unique by the presence of calcite. Except for the order Rhodogorgonales, a tropical group of gelatinous algae with calcification limited to calcite husks, the remaining orders are diverse and widely distributed, characterized by having calcified cell walls and highly variable morphology. Recent application of molecular phylogenies fostered our understanding of their affinities within the Florideophyceae; nevertheless, the origin and evolution of this group are still controversial. Unequivocal coralline fossils date back to the early Cretaceous (140 Mya), however, a series of fossils with some coralline algal resemblance would push back the origin of the group to the Neoproterozoic (Ediacaran, ca. 600 Mya). The taxonomic affinities of these fossil taxa however is not without discussion. In the present study, we provide a comprehensive phylogenetic framework, enabling the reconstruction of the evolutionary history of the subclass Corallinophycidae. This approach involved phylogenetic analyses of molecular data obtained for seven genes (*cox1*, *psbA*, *rbcL*, 23S rRNA, SSU rRNA, LSU rRNA and EF2), and the revision of available literature on taxonomy, phylogeny and systematics for all the genera currently accepted taxonomically.

An exhaustive list of fossil records (>2000) encompassing different genera was compiled in order to obtain the most accurate timeline for calibrating the Corallinophycidae phylogeny. Our main results support an origin of the subclass Corallinophycidae in the Lower Jurassic (Toarcian, *ca.*180 Mya), followed by the separation of two main lineages, Rhodogorgonales and Sporolithales, in the Upper Jurassic and the split of the orders Hapalidiales and Corallinales in the lower Cretaceous together with the emergence of the monotypic *Corallinapetra*.

3OR.7

PRONOUNCED GEOGRAPHIC STRUCTURING AND ENDEMISM IN FRESHWATER DIATOMS OF THE ANTARCTIC REALM

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Microorganisms are crucial players in all global biogeochemical cycles and ecosystem functioning in terrestrial and aquatic habitats. Despite this, information on their large-scale biogeographic structuring is largely lacking, mainly due to a lack of taxonomic resolution and consistency in the available datasets. Despite a growing number of morphology- and DNA-based studies on microbial biodiversity, our understanding of large-scale microbial biogeographical patterns remains a contentious issue, mainly because of the lack of taxonomic resolution and consistency in the available datasets. In this lecture, an analysis will be presented of biogeographic patterns in freshwater diatoms based on a high-resolution and internally fully consistent species-level taxonomic data set from > 400 lakes covering the entire

Antarctic Realm. A strong biogeographic structuring at multiple spatial scales was observed with distinct, differently sized diatom floras characterizing Continental Antarctica, Maritime Antarctica and the Sub-Antarctic islands. Additional biogeographic provincialism emerged in all three regions. These patterns were underlain by species turnover rather than nestedness; explained predominantly by historical and spatial factors, such as distance between regions and differences in the deglaciation history. A total of 59% of the recorded species are currently only known from the Antarctic Realm. The proportion of regionally restricted species was particularly high in predominantly terrestrial genera and, in contrast to the local and regional richness, significantly increased with increasing latitude. This strong biogeographical structuring suggests that effective dispersal between the biogeographical regions has been limited, fostering the evolution of highly endemic diatom floras, making a compelling case for the important role of historical events in the evolution of lacustrine diatoms, which is similar to macroscopic organisms from the Antarctic Realm based on morphological and genetic data.

3OR.8

BRIDGING FUNCTIONAL AND PHYLOGENETIC DIVERSITY OF MARINE HETEROTROPHIC PROTISTS VIA SINGLE-CELL TRANSCRIPTOMICS

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The comprehensive description of unicellular heterotrophic protists is essential for understanding the functioning of marine ecosystems and defining evolutionary relationships within marine microbial communities. For that reason, new insights into the functional genes of key protists, such as ciliates and dinoflagellates, are needed to complement the increasing taxonomic complexity and bridge the gap

between various eco-functional processes in the ocean. In this study, single-cell transcriptomic sequencing proved to be an efficient method to create a snapshot of expressed genes of unicellular heterotrophs. We sequenced 65 single-cell transcriptomes from 20 fresh field samples collected from Sub-Arctic and North Sea waters. These 13 ciliate and 52 dinoflagellate transcriptomes will generally contribute to a greater understanding of functional and evolutionary processes of these marine protists. Further, we generated multi-gene phylogenies of several dozen genes to unravel the relationships of these heterotrophic taxa to other dinoflagellates and ciliates, respectively. These approaches also helped to elucidate the evolution of functional genes and traits for these understudied essential groups. Additionally, the datasets were incorporated into our metatranscriptomic reference database to fill the gap (of approx. 50%) of genomic information of heterotrophic organisms and their functional processes. Overall, identifying the phylogenetic relationships and functional diversity of heterotrophic and mixotrophic protists will clarify paramount marine microbial food web processes and provide clues to the system's sensitivity to climate change.

3OR.9

STUDIES ON THE BIODIVERSITY OF ARCTIC PROTISTS – BRIDGING THE GAP BETWEEN MORPHOLOGICAL AND MOLECULAR APPROACHES

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To understand the functioning of the Arctic ecosystem and predict its response to the ongoing climate warming, it is essential to map the diversity and distribution patterns of Arctic protists. Recent metabarcoding surveys in the Arctic Ocean have revealed a remarkable diversity of protist OTUs. However, these OTUs could not always be assigned to the taxonomically verified morphospecies due to inadequate representation of morphologically described Arctic protists in current reference DNA sequence databases. The TaxMarc project aims to bridge this gap by applying state-of-the-art molecular and

morphological approaches to create a taxonomic framework for studying Arctic protist diversity and distribution. In recent years, we have conducted a number of sampling surveys in the Atlantic section of the Arctic focusing on Svalbard, the Barents Sea, and the Arctic Ocean, with an aim to comprehensively describe the protist diversity. In these surveys, the metabarcoding of protist communities was coupled with detailed morphological analysis using light and electron microscopy, thus linking morphological traits with molecular diversity. Moreover, an intensive culturing effort which included highly understudied sea-ice taxa led to the morphological and genetic characterization of over 200 new microalgal cultures, mostly diatoms and dinoflagellates. This allowed for the expansion of rRNA gene reference databases, descriptions of new species and advances in phylogenetic placements of these taxa. Subsequently, single-cell PCR of mostly heterotrophic dinoflagellates and ciliates generated almost 100 new and morphologically verified 28S and 18S rRNA reference sequences from the Barents Sea and the Arctic Ocean, which will greatly improve the ability to detect these taxa in metabarcoding datasets. In conclusion, our work will lay the foundation for future metabarcoding and microscopy surveys of protist diversity and distribution in the Arctic and at the same time advance the knowledge on phylogeny and taxonomy of modern protists.

3OR.10

ARE SEAWEEDS FROM THE MEDITERRANEAN SEA AND ATLANTIC OCEAN SISTER?

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The Mediterranean Sea is the cradle of a wide diversity of marine organisms. Among them, c.a. 1,200 species of seaweeds are currently recognized belonging to the three phylogenetically distinct lineages of seaweeds (green, red and brown algae). In comparison with other marine organisms, the endemism rate of algae is rather low. On the basis of our extensive recent collections from both the Atlantic and the Mediterranean European coasts we used the tools of molecular systematics (5'end of CO1) to examine the relationship between Atlantic and Mediterranean species of brown and red algae. The phylogeographical

patterns of Mediterranean marine flora revealed by molecular systematics tools suggest a significant flow of Atlantic ocean species into the Mediterranean Sea through the Gibraltar opening. Nonetheless, a few lineages without close allied detected anywhere in the world have been highlighted. The implications of these results will be discussed in terms of conservation and biodiversity dynamics in the context of global change. Last but not least, we will examine the possible causes of the underestimation of the algal diversity of the Mediterranean Sea in light of the history of the phycology of this region.

3OR.11

SPECIES COMPOSITION OF EPIPHYTIC DIATOMS CONFIRM MARINE BIOGEOGRAPHICAL PROVINCES IN SOUTH AFRICA

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Regularly sampling since 2012 of diatom assemblages along the entire coast of South Africa from Sodwana Bay (Indian Ocean) to Porth Nolloth (Atlantic Ocean) and sporadically to Terrace Bay in Namibia provided a valuable spatial and temporal dataset. The sampling was accompanied with measurements of basic environmental variables that included salinity, temperature, pH, oxygen and, in the last two years, also nutrients. Various substrates and environments were sampled including sand, mud, rock scrapes, plankton, seaweeds and seagrass. From 31 sites we sampled and analyzed diatom assemblages from *Corallina* sp. and *Porphyra* sp. (10 sites), *Ulva* sp., *Codium* sp., *Halimeda* sp. (13 sites), unidentified Phaeophyta (7) and *Zostera* sp. (1). Dominant epiphytic diatoms in our counts were: *Amphora helenensis*, *Cocconeis scutellum*, *C. californica*, *Nitzschia frustulum*, *N. peridistincta*, *Rhoicosphenia flexa* and *Tabularia fasciculata*. The

spatial diversity of the diatom assemblages and their relation to substrate have been tested with multivariate analysis and multimetric ordination analysis (nonparametric multidimensional scaling – MDS). This grouped the diatom assemblages according to the sampling location. Sampling sites from the West and East coasts formed two distinct clusters with the warm temperate South coast in an intermediate position between the cold west and warm east coast. The Langebaan Lagoon site was firmly nested within the East coast cluster. We hope that the current result will serve as a baseline for the future assessment of the impact of ongoing climate change along the South African coast.

3OR.12

HIDDEN GENERA WITHIN THE OSCILLATORIALES IN POLAR REGIONS

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Recently, the very thin filamentous cyanobacteria have undertaken a significant taxonomical evaluation. These species are usually ubiquitous in a wide range of environments. They were traditionally studied according to their morphological properties only. With the introduction of additional taxonomic methods (cytomorphological analyses, molecular sequencing, exact ecological studies, etc.) traditional genera such as *Leptolyngbya*, *Pseudanabaena*, *Limnothrix* and *Phormidium* were found to be polyphyletic. Nowadays, the new genera are described worldwide, including the Polar Regions. These taxonomical changes greatly influence biogeographical studies. Recent studies show, that some species previously described as endemic, could have a worldwide or circum-polar distribution. Presented research collected data about microbial phototrophs from Petuniabukta, Svalbard and North Slope, Alaska during a summer research expeditions in the period of 2011-2017. They are focused on filamentous cyanobacterial genera from terrestrial and water biotopes, where they represent a dominant species of phototrophs. The results bring descriptions of new species and genera specific for these biotopes, but they also bring evidence about the worldwide distribution of some species, which were previously considered an endemic.

3OR.13**BIOGEOGRAPHY AND ECOLOGY OF THE CHLOROMONADINIA PHYLOGROUP (CHLOROPHYCEAE)**

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The phylogroup *Chloromonadinia* encompasses a high diversity of unicellular green flagellates reported mainly from freshwaters and snow. However, very little is known about (aero)-terrestrial members of the *Chloromonadinia*. We have recognized four validly described genera and numerous species *incertae sedis* within the phylogroup. The most widespread and diversified genus is *Chloromonas*, occurring in both freshwater and terrestrial environments. *Ixipapillifera* also inhabits both environments, whereas *Gloeomonas* occurs only in freshwaters and *Chlainomonas* represents snow algae. The phylogroup *Chloromonadinia* is known for its extremophilic and extremotolerant members and our aim was to contribute to the knowledge of their distribution and phylogeny. We have studied several new and authentic *Chloromonas*-like strains, isolated from polar and temperate habitats. Our results have shown that ecologically similar species cluster close to each other within the *Chloromonadinia* phylogeny. As an example, the genus *Chloromonas* consists of three monophyletic lineages supported by morphological as well as ecological differences. *Chloromonas* clade 1 (with the type species *Cr. reticulata*) encompasses mesophilic and psychrotolerant species that likely do not form snow blooms. In contrast, *Chloromonas* clade 2 includes psychrophilic snow species and clade 3 covers mesophiles. Other lineages of the phylogroup can be found also in acidic environments and we have reported such species for the first time in a terrestrial habitat. From the biogeographic point of view, the phylogroup encompasses several species with a widespread distribution. On the other hand, numerous species are known only from a single locality, especially the majority of psychrophilic snow species. However, the latter pattern can be biased by limited and unevenly distributed sampling efforts.

3OR.14**EVOLUTION OF SULFUR METABOLISM IN PHYTOPLANKTON**

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The concentration and chemical state of sulfur (S) in oceans has varied substantially during Earth's history and may have played a central role in the evolution of phytoplankton communities. Diatoms, coccolithophorids and dinoflagellates arose during an interval particularly marked by increased sulfate concentration in seawater and became major primary producers in oceans, whereas abundance of green algae and cyanobacteria diminished in the same period. The physiological mechanisms leading to this transition are still unclear. Although sulfate assimilation is assumed to be highly conserved in all photosynthetic organisms, some recently published particularities, especially among algae groups, may help understanding differential growth and competitiveness under varying sulfate concentrations. The first committed step of sulfate assimilation seems to host most of these peculiarities on such parameters as regulation, activity and localization. This step consists in the activation of sulfate, producing adenosine-5'-phosphosulfate (APS) and is catalysed by the enzyme ATP sulfurylase (ATP-S), which, for instance, appears to be subject to redox regulation in oceanic cyanobacteria and eukaryotic algae (except dinoflagellates), but not in plants, freshwater and coastal cyanobacteria and dinoflagellates. Also, this enzyme's activity and localization have been described as fairly heterogenous among clades. Our study aims to raise comprehension about the transitional period when red lineage algae (chlorophyll *a+c*) overcame green lineage algae (chlorophyll *a+b*) concomitantly to a considerable increase of sulfate in seawater. We hypothesize that ATP-S heterogeneity could be the major physiological factor affecting phytoplankton composition in this case. To investigate it, microalgae belonging to different groups will be S starved and assessed in terms of growth rate, final cell yield, C, N and S quotas and ATP-S activity and regulation.

3OR.15**SEAWEED PHYLOGEOGRAPHY IN THE NORTHWEST PACIFIC**

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Coastal tectonic configurations during the Quaternary ice ages combined with substantive oceanographic processes (e.g. surface current systems) in the Northwest Pacific enable it to be an important centre of seaweed species diversity on the earth. However, phylogeographic patterns and processes of seaweed diversity in this area just have received limited research attention since 2010s, relative to the continuous and intensive research effort in the North Atlantic in the past few decades. Here, we summarized the main research advances of molecular phylogeography and biodiversity conservation of seaweeds in the Northwest Pacific during the past a few years, including results from other research units in East Asia. We focused on the brown seaweeds *Sargassum*, the red seaweed *Chondrus* and the green seaweed *Ulva*. We concluded that: i) sea level fluctuations induced by the Quaternary ice ages imposed significant signatures in contemporary seaweed populations, leading to diverged genetic lineages; ii) ocean surface circulations drove population genetic homogeneity and proximity of distant populations and/or lineages; iii) some late-Pleistocene glacial refugia identified, including the Japanese Islands from Kyushu and Shikoku to the Kanto district, the southwest of Hainan Island, China; iv) phylogeographic diversity provided important insights for conserving commercially cultivating seaweeds species. We also discuss the main problems existed in seaweed phylogeography in this area and possible resolutions to better understand diversity and evolution of seaweeds in the Northwest Pacific.

3OR.16**CRYPTIC DIVERSITY IN THE WIDESPREAD INTERTIDAL RED ALGAE, *GLOIOPELTIS FURCATA* (GIGARTINALES, RHODOPHYTA) FROM THE NORTHWESTERN PACIFIC**

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The genus *Gloiopeletis* is known as commercial uses because it is an edible red alga used as a thickening agent, agarose named funoran. Since the economic value of seaweeds is species-dependent, it is essential to obtain accurate species-level identification in the genus *Gloiopeletis* which is absent an obvious morphological differences between species. In this study, we aimed at characterizing the molecular diversity of the widespread intertidal red algae, *Gloiopeletis furcata*, from Korea and Japan using the mitochondrial COI barcoding region to investigate the phylogeographic patterns based on haplotype networks. The phylogenetic analyses provided the evidence of 10 cryptic lineages within *G. furcata*, highly divergent clades. Distribution map of haplotypes revealed that *G. furcata* populations along the Nagasaki in Japan and Jeju Island in Korea exhibited the highest genetic diversity and the areas might be the diversification center of *G. furcata*. Lineage A was found only in the cold temperate region (eastern Korea and Hokkaido in Japan), whereas lineage C was mostly distributed in the warm temperate region from the southern part of Japan and Jeju in Korea. This study highlights that *G. furcata* shows strong genetic heterogeneity at the cryptic species level and extensive geographical overlap. We will provide descriptions of several new *Gloiopeletis* species in the near future.

3OR.17**SPECIES DIVERSITY, ENDEMISM AND GENETIC STRUCTURE IN THE BANGIALES (RHODOPHYTA) ALONG THE SOUTH AFRICAN COAST**

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The taxonomy of the Bangiales has seen a major paradigm shift over the past two decades. The order, which previously consisted of two genera based on morphology, now comprises sixteen genera based on DNA sequence data. The present study explored the biodiversity of the Bangiales in South Africa, where six species, belonging to three genera, have been recorded. Based on a comprehensive collection of the Bangiales from the region, 241 sequences were generated from three unlinked loci (nSSU, *rbcL* & *cox1*). Species were identified or delimited using an integrative taxonomic approach based on molecular, morpho-anatomical and ecological data. A multigene phylogeny (nSSU, *rbcL* & *cox1*) was reconstructed and three DNA-based species delimitation methods (ABGD, GMYC, PTP) were applied to the mitochondrial, *cox1* and the plastid, *rbcL* genes. Results indicate that 16 Bangialean

species in three genera (11 *Porphyra*, four *Pyropia* and one *Bangia*) are present in South Africa. Two afro-temperate species were described, *Pyropia meridionalis* sp. nov. and *Porphyra agulhensis* sp. nov. and two species with uncertain taxonomic status are yet to be confirmed using a molecular approach. Species boundaries were affirmed for two endemic species, *Py. saldanhae* and *Py. aeodis*, but not for *Porphyra capensis* which was shown to comprise multiple cryptic species. High genetic diversity and geographic structure was apparent within and among species of *Porphyra* in the region. Globally, South African Bangiales shared close phylogenetic affinities to species from the Southern Hemisphere and provide support for historic connectivity in the Southern Ocean. The extensive genetic diversity and endemism found along this coastline compares well with other Southern Hemisphere countries.

4. Algal cell biology: making sense of cell structure

4KN.1

TERRESTRIALIZATION IN THE STREPTOPHYTE *ZYGNEMA*: INVOLVEMENT OF CELL WALL COMPONENTS AND TRANSCRIPTOMIC CHANGES

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The conjugating green alga *Zygnema* sp. thrives in extreme habitats such as alpine and polar freshwater springs, where it develops large mats by vegetative cell division in early spring. These mats change during the season and pre-akinetes develop from vegetative cells, which strongly increases their resistance against different abiotic stresses. We investigated the role of homogalacturonan (HG, major pectic domain) as a key agent of resistance against water scarcity and the involvement of arabionogalactan proteins (AGPs) in cell adhesion phenomena. Older akinete-rich filaments produced more homogalacturonan, resulting in a higher desiccation resistance as monitored by measurements of the effective quantum yield of photosystem II. AGPs are most likely key adhesion molecules, allowing algae to interact with their substrates and neighboring filaments. For transcriptomic analysis, field samples of *Zygnema* sp. were collected in the high arctic (Longyearbyen, Svalbard). The bottom layer of the extracted mats contained dark green algal filaments and a top layer contained pale filaments. This layer was directly exposed to high levels of irradiation. In the top layer, 6,569 genes were significantly up-regulated, 149 down-regulated. Up-regulated genes include compounds of PS I and II, light-harvesting complexes and chlorophyll synthesis, and early light-inducible proteins (ELIPs), carbohydrate metabolism including starch degrading enzymes and cell wall metabolism proteins. These observations show an active adaptation process in this sister group to land plants that likely contributes to terrestrialization.

4KN.2

NEW STUDIES ON THE WARNOWIACEAE (DINOPHYCEAE), SOME OF THE MOST EXTRAORDINARY MARINE FLAGELLATES KNOWN

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The warnowiaceans constitute a family of unicellular, gymnodinioid flagellates characterized by a unique and complex eye-like photoreceptor in the cell, the ocelloid, which includes both a lens-like structure and a photoreceptor lined by a cup-shaped shield of carotenoids. Among the ca. 6 genera, species of *Nematodinium* are unique by possessing also normal-looking chloroplasts and so-called nematocysts, each nematocyst resembling a rocket battery. Species of *Proterothropsis* differ by lacking chloroplasts, while the remaining 4 genera are thought to lack both chloroplasts and nematocysts. Description of the first species of ocelloid-bearing dinoflagellates, in Germany in 1884, was received with disbelief, and it was argued that such a creature did not exist: it was claimed to be a protozoan that had been eating part of a medusa at the time it was fixed in osmium tetroxide. However, some 50 species of the family have now been described, although the taxonomy is in a state of chaos, mostly because cells are very fragile and prone to change when handled. I will report on our ongoing studies on members of the Warnowiaceae from Denmark and Greenland, which include serial sectioning of whole cells (as many as 600 sections through each cell), from which we aim to elucidate the detailed structure of the ocelloid, the nematocysts, the flagellar apparatus, etc. If progress permits, I may also be able to report on studies on eyespot function, presently underway by an associated group at the universities of Copenhagen and Lund. At the end of the project we hope to understand more about the function of the cell's organelles and also to use a modern approach to evaluate how to create

a reliable taxonomy of at least some of the family's species.

4OR.1

NANO X-RAY FLUORESCENCE TOMOGRAPHY (SR-NXRF) FOR IMAGING ELEMENT DISTRIBUTION AT THE SUBCELLULAR LEVEL IN ALGAE

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For understanding many aspects of metabolism – especially involving inorganic element such as metals and halogens – accurate information about the localization of transport and storage is essential. Many conventional techniques are prone to artefacts during sample preparation: (1) Chemical – chemical speciation has changed due to sample treatment (disruption of organelles, impact of fixatives, etc.). (2) Dislocation – the localization observed is not the original/natural one. In this context, synchrotron-based nano X-ray fluorescence tomography (SR-nXRF) offers significant new opportunities. By eliminating the need for chemical fixation and freeze-drying of biological samples, all the liquid and dissolved content of cells as well as membranes are preserved intact in their original location. A well-known phycological model, the accumulation of iodine in *Laminaria digitata*, is used to demonstrate the usefulness of this approach for the first time ever in an algal model, and to provide groundbreaking new insight into the mechanism of the storage mode of iodine in seaweeds, with implications for the understanding of innate immunity and the role of seaweeds in atmospheric and marine chemistry.

4OR.2

EVOLUTION OF THE MIP (AQUAPORIN) PROTEIN FAMILY WITHIN THE VIRIDIPLANTAE: A STORY OF GAINS AND LOSSES

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Aquaporins are water channels facilitating the water flow through biological membranes. They are part of a large protein family called “Major Intrinsic Proteins” which facilitates membrane transport of water and other non-charged molecules such as glycerol. Embryophyte genomes encode a large number

(>20) of MIPs forming several subfamilies often localized to specific compartments (e.g. PIPs localized to the PM; TIPs found in the tonoplast, or SIPs present in the ER membrane). In contrast, the sequenced green algal genomes contain only a small number (<6) of MIP protein family members. In this presentation, we will present a phylogeny of the MIP protein family in the Viridiplantae showing several gains and losses of complete subfamilies. Driving forces for the complex evolution pattern within the Viridiplantae and the importance of this protein family for the evolution of land plants from streptophyte algae will be discussed.

4OR.3

DIFFERENTIAL INVOLVEMENT OF CELLULAR MACHINERY IN PHOTOMOVEMENT OF *SPIROGYRA VARIANS*

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Freshwater algae living in shallow waters have evolved various photomovement to optimize their photosynthetic efficiency. The filaments of *Spirogyra varians* exhibit complex photomovement and form a compact mat which enables them to adjust their light exposure. Different patterns of movement were observed in response to monochromatic lights. The filaments of *S. varians* showed positive phototropism under blue light. Under red light, the filaments bent to the irregular shape, but rapidly became unbent by exposure to far-red light. To decipher the genetic control of photomovement, phototropin homologues (*SvPHOTA*, *SvPHOTB*) and phytochrome homologues (*SvPHY1*, *SvPHY2*, *SvPHYX2*) were characterized in *S. varians*. Photomovement of filaments was analyzed using cytoskeleton inhibitors under monochromatic lights. The movement of filaments under red light was inhibited by the treatment of the microtubule inhibitor, oryzalin. The actin inhibitor, cytochalasin D did not show any inhibitory effect. The blue light responsive movement, however, was blocked only when the filaments were treated with the combinations of cytochalasin D and oryzalin. In immunofluorescence staining, the formation of actin filaments in *S. varians* differently induced by monochromatic lights. Mannitol and sorbitol treatment significantly inhibited far-red light movement suggesting that turgor pressure is the driving force of this movement. Our results suggested that the complicated photomovement of *S. varians* is the result of photoresponse driven by different combinations of cellular machinery.

4OR.4**REACTIVE OXYGEN SPECIES (ROS) SIGNAL TRIGGERS FERTILIZATION IN A RED ALGA *BOSTRYCHIA MORITZIANA***

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The fertilization of red algae offers a good model for the studies on cell-cell recognition, signal transduction and the relocation of cellular organelles. The fertilization process of *Bostrychia moritziana* composed of five distinctive stages; 1) binding between male spermatia and female trichogyne, 2) spermatial nuclear division, 3) hydrolysis of trichogyne wall, 4) formation of fertilization channel between spermatia and trichogyne, 5) migration of spermatial nuclei in the trichogyne and gamete nuclear fusion. Gamete binding in red algae is mediated by a lectin-carbohydrate complementary system, and female lectins have been isolated and molecularly characterized. However, little is known for the signal transduction after the gamete binding. Our histochemical studies using 3,3-Diaminobenzidine (DAB) staining showed that the accumulation of hydrogen peroxide occurs successively after gamete binding; first at the binding area between spermatia and trichogyne, then at the cytoplasm of trichogyne and carpogonial branches. When male gametophyte was pretreated with diphenylene iodonium (DPI), a suicidal inhibitor of NADPH oxidase for 30 min, the released spermatia could still bind to the trichogyne, but no ROS accumulation was observed at the binding area as well as the carpogonial cells. Quantitative PCR and RNA *in situ* hybridization results showed that significant upregulation of NADPH oxidase occurred in the spermatia and female gametophyte after the gamete binding. The development of carposporophyte was significantly inhibited when the spermatia were pretreated with DPI. These results indicate that successive ROS signal transduction occurs from spermatia to trichogyne and then to carpogonial cells, and this signal is essential for the successful fertilization and successive development of carposporophyte in *Bostrychia moritziana*.

4OR.5**CELL REPAIR AND INTERCELLULAR NUTRIENT TRANSFER IN FILAMENTOUS RED ALGAE, *GRIFFITHSIA MONILIS* AND *HETEROSIPHONIA JAPONICA***

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Cell repair needs help from adjacent cells and transportation of nutrients and signals among cells is essential to maximize benefit for being multicellular. It has long been believed that red algal intercellular transport is “plugged” by the pit connection which is the only physical connection between adjacent cells. We hypothesized that pit connection is a specialized junction for intercellular transport analogous to plasmodesmata of other plant and algae. The intercellular transport was examined by using microinjection of fluorescent tracers to various cell types of two filamentous red algae, *Griffithsia monilis* and *Heterosiphonia japonica*. Fluorescein isothiocyanate-dextran (FD) with different molecular sizes and a recombinant green fluorescent protein were used as tracers. The limit size of molecules which can be transported to the next cells was in between 20 kDa to 40 kDa. The time necessary for the transport to the next cell was dependent on the molecular size and the direction of the transport. The fluorescent dextran of 3-10 kDa was transported to next cell in 1-2 h, but that of 20 kDa took 24 to 48 hours. Often, the low molecular weight tracers were transported to whole cells in a filament in a day. The fluorescent tracer over 40 kDa was not transported to the next cell, but was strongly accumulated in the pit-connection area. The transportation occurred faster to adjacent reproductive cells and to the apical direction than to the vegetative basal cells. Our results showed that pit-connection plays a key role as a transfer junction in red algal cells and macromolecules can travel across the cells by leaking through the gap between the pit-plug and cell wall.

4OR.6**BIOVOLUME CALCULATION FOR PLANKTONIC ALGAE BY USING 3 DIMENSIONAL MODELS**

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Biovolume is one of the most important characteristics of planktic organisms which basically influences their functional properties like sinking velocity or tolerance to grazing. Biovolume calculation of microalgae is based on the determination of the geometrical shapes that approximate best the shape of the algal cells. After measuring the necessary dimensions of the cells and using the formulas for the geometric shapes cell biovolumes can be calculated. However, this approach may have uncertainties in

the case of complicated forms. Here we propose a new approach which uses 3D models to visualize algae, and calculates the volume and the surface of these virtual models. On the bases of these models, specific shape and surface area constants could be calculated. These species-specific constants can be stored in a database, and enable a quick and accurate surface area and volume computation even for the most complicated algal forms. Precise estimation of these metrics could help in the calculation of size-related metrics and traits like surface area/volume ratio or diameter of spherical equivalent, which allows more accurate data analysis and interpretation of the results in phytoplankton ecology.

5. Life cycles and life strategies of micro- and macroalgae

5KN.1

EVOLUTION OF LIFE-CYCLE-RELATED DEVELOPMENTAL PROCESSES IN THE BROWN ALGAE

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The molecular processes that regulate life cycle progression provide an essential foundation for the correct initiation of diverse biological processes, including multicellular development and sexual reproduction¹. The life cycle of the model brown alga *Ectocarpus*²⁻⁴ involves an alternation between two independent multicellular organisms, the sporophyte and the gametophyte. We have shown that the identities of the two generations are not determined by ploidy, but rather are determined genetically. Mutations affecting either the *OUROBOROS* (*ORO*) or the *SAMASARA* (*SAM*) locus result in complete conversion of the sporophyte generation into a -gametophyte⁵. Both *ORO* and *SAM* encode three amino acid loop extension homeodomain transcription factors (TALE HD TFs). Similarities between *ORO/SAM* and HD-TF-based life cycle regulation systems in other eukaryotic supergroups indicate not only that these systems have an extremely ancient origin but also that TALE HD TFs have been independently recruited to regulate sporophyte developmental programs in at least two different complex multicellular eukaryotic supergroups, Archaeplastida and Chromalveolata. The presentation will discuss genetic regulation of life cycle progression and will also look at some of the genetic and epigenetic events that occur downstream of the switch to either the sporophyte or gametophyte developmental programs.

5KN.2

LIFE CYCLE COMPLEXITIES OF THE BLOOM-FORMING COCCOLITHOPHORE *EMILIANA HUXLEYI*

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Emiliana huxleyi is a prevalent microalga that forms extensive blooms in high latitude regions of the oceans with major ecological and biogeochemical implications. *E. huxleyi* has been shown to alternate between the bloom-forming calcified diploid phase, and a less known biflagellate non-calcified haploid phase as part of a haplodiplontic sexual life cycle. Recently we have additionally shown that under infection by specific lytic viruses (EhV) that are responsible for the termination of blooms, a subpopulation of diploid-calcified cells that are sensitive to EhV can produce viral-resistant cells that display a biflagellate phenotype like haploid cells, but retain a diploid level. These new phenotype-ploidy uncoupled cells may be produced as a mechanism to escape EhV and enhance survival rates during blooms at sea. In order to advance our understanding of the degree of differentiation between calcified-diploid, haploid and uncoupled cells as well as the potential drivers of phenotype switch during EhV infection, we compared the transcriptome profiles of the 3 cell types. We found that at the gene expression level, haploid and decoupled cells are more similar relative to the calcified cells, and we were able to unveil part of the genetic foundation underlying the observed phenotypic variability. Moreover, we developed of life-phase specific gene-markers that we can now use to probe different life cycle phases of *E. huxleyi* at sea. Overall our study emphasises the complexity of the life cycle of *E. huxleyi* and the adaptive significance of morpho-physiological plasticity in eukaryotic microbes.

5OR.1**DIFFERENCES IN ACID-BASE REGULATION BETWEEN THE HEAVILY CALCIFYING DIPLOID AND THE WEAKLY CALCIFYING HAPLOID LIFE CYCLE STAGE OF THE COCCOLITHOPHORE *COCCOLITHUS BRAARUDII***

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Coccolithophores are calcifying microalgae that carry characteristic calcite platelets (coccoliths) on their surfaces. Most coccolithophore species exhibit diploid and haploid life cycle stages, each adjusted to different environmental conditions. The diploid life cycle stage of the coccolithophore *C. braarudii* is heavily calcifying with calcification rates that exceed the rates of photosynthesis. During calcification, it produces excess amounts of H⁺ that have to be continuously disposed by voltage-gated proton channels in order to keep the intracellular pH constant (Taylor et al. 2011). The haploid life-cycle of *C. braarudii* is weakly calcifying and therefore generates significantly less H⁺. This life-cycle stage may therefore use substantially different physiological strategies to regulate their intracellular pH under varying environmental conditions. The aim of this work is to identify the differences in the cellular mechanisms involved in pH regulation between both life-cycle stages. We combine physiological measurements with molecular and pharmacological approaches, allowing to identify and characterize membrane proteins involved in pH homeostasis.

5OR.2**WINTER SURVIVAL CAPACITY OF MICROSCOPIC POLAR KELP STAGES**

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In October 2016, we initiated an experiment simulating the seasonal sequence of autumn (short daylengths, low light), Polar winter (no light) and spring conditions (short daylengths, low light) at Kongsfjorden/Spitzbergen under current and elevated winter temperature scenarios (0, 4 and 8°C) in the laboratory. We compared the development of spores of three sympatric kelp species (*Saccharina latissima*,

S. nigripes, *Laminaria digitata*), their survival capacity, gametogenesis and sporophyte recruitment and hypothesized that warming scenarios will negatively affect winter survival. Under autumn conditions, spores preferably developed into multi-cellular gametophytes, irrespective of the species and only rarely formed microscopic sporophytes within a 3-week period. In contrast to our hypothesis, uni- and multi-cellular gametophytes of all three kelp species had a high ability to survive 4 months of darkness irrespective of temperature. Dark survival capacity of microscopic sporophytes, however, was considerably reduced at 8 °C compared to 4 and 0°C. Post-cultivation simulating spring conditions after Polar night conditions resulted in highest sporophyte recruitment when multicellular gametophytes had entered the dark period. There were species-specific differences in recruitment capacity dependent on temperature. While cold-temperate to Arctic *S. latissima* recruited best at 8°C, Arctic *S. nigripes* recruited best at 0°C. *In situ* sporophyte recruitment of the three kelp species was followed in parallel on seeded field tiles but was extremely slow, and macroscopically visible juvenile sporophytes became only apparent in August 2017 - 10 months after seeding. The overall results suggest that formation of multi-cellular gametophytes is a natural mean of enhancing recruitment success and that there is a very good adaptation of Arctic kelp gametophyte stages to the seasonal day-length regime and Polar darkness, but future elevated winter temperatures may change the spring recruitment potential of kelps.

5OR.3**SEXUAL REPRODUCTION IN *ULVA* - HOW DOES IT LOOK?**

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The green algae of genus *Ulva* are among the most common algal group of the shallow marine bottom. Plenty of publications cover their life history and demonstrate a wide range of variation in their sexual and asexual reproduction. Those studies were mostly made in laboratory conditions while just a few included field observation that mainly investigated the connection between lunar phases and the development of fertile plants. Based on *in situ* observation, we discovered how sexual reproduction in several *Ulva* species is highly synchronized events. Each of the species releases gametes at a species-specific intensity of light around sunrise. In the semi-field experiment, made in parallel with *in situ* observation,

we determined the exact time the gametes were released, in each of the observed *thalli*. Every fertile gametophyte releases gametes within a few minutes and contributes to develop the visible green gamete cloud. Depending on the algal density and sea state condition, gamete cloud can reduce visibility in the sea to zero and can last even over an hour. In such long-lasting reproduction event, gamete cloud is usually a result of reproduction in several taxa that occurred successively around sunrise. Synchronized release of gametes in sessile species is essential to ensure successful fertilization. Although synchronized reproduction was predicted in *Ulva* species, the level of their synchronization, the quantity and visibility of the gamete cloud is much greater than expected.

5OR.4

THE BHLH-PAS PROTEIN RITMO1 REGULATES DIEL BIOLOGICAL RHYTHMS IN THE MARINE DIATOM *PHAEODACTYLUM TRICORNUTUM*

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Periodic light-dark cycles govern the timing of basic biological processes in organisms inhabiting land as well as the sea, where life evolved. Although prominent marine phytoplanktonic organisms such as diatoms show robust diel rhythms, the mechanisms regulating these processes are still obscure. By characterizing a *Phaeodactylum tricornutum* bHLH-PAS nuclear protein, hereby named RITMO1, we shed light on the regulation of the daily life of diatoms. Alteration of RITMO1 expression timing and level results in lines with deregulated diurnal gene expression profiles compared to the wild-type cells. Reduced gene expression oscillations are also observed in these lines in continuous darkness, showing that the regulation of rhythmicity by

RITMO1 is not directly dependent on light inputs. We also describe strong diurnal rhythms of cellular fluorescence in wild-type cells, which persist in continuous light conditions, indicating the existence of an endogenous circadian clock in diatoms. The altered rhythmicity observed in RITMO1 over-expression lines in continuous light supports the involvement of this protein in circadian rhythm regulation. Phylogenetic analysis reveals a wide distribution of RITMO1-like proteins in the genomes of diatoms as well as in other marine algae, which may indicate a common function in these phototrophs. This study adds new elements to our understanding of diatom biology and offers new perspectives to elucidate timekeeping mechanisms in marine organisms belonging to a major, but under-investigated, branch of the tree of life.

5OR.5

EVOLUTION OF MATING TYPES IN MAMIELLOPHYCEAN GREEN ALGAE GENOMES

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Sex is a ubiquitous and ancestral feature of eukaryotes that is well documented or predicted to occur in many protists lineages. Nevertheless, direct observation of sexual interactions is lacking for many unicellular eukaryotic species, as is the case for Mamiellophyceae, a class of planktonic green algae. These worldwide spread algae contain the smallest photosynthetic eukaryotes and form the basis of the food chain in coastal marine ecosystems. In natural populations of *Ostreococcus tauri* from the order Mamiellales, indirect genetic evidence of recombination suggests that a cryptic sexual stage for this genus exists. In many protists, sexual interactions are determined by mating types (MTs), sex chromosome-like regions that may have evolved before the development of morphologically different gametes. Recently, our group described a candidate MT locus in *O. tauri* located in a 500 kb long region on the Big Outlier Chromosome (BOC) with low GC (guanine-cytosine)

content, accumulation of transposons and recombination suppression, that taken together suggest that this is a sex chromosome. Here, we use eight available Mamiellophyceae genomes, 33 transcriptomes from the “Marine Microbial Eukaryote Transcriptome Sequencing Project” (MMETSP) and the “1000 plants project” (1KP), to address two main questions regarding these genomic regions: what is the evolutionary history of candidate MT loci in the Mamiellophyceae, and is there evidence for MTs in other species of this group? Phylogenomic and compositional analysis of specific, shared and core gene families located in MT loci and low GC regions of *Ostreococcus*, *Bathycoccus* and *Micromonas* suggest an ancient divergence of two MTs in the genus *Ostreococcus*, while transcriptomic data moreover indicates the presence of candidate MTs in *O. lucimarinus*, *O. mediterraneus* and possibly *M. commoda*.

5OR.6

SEXUAL AND ASEXUAL REPRODUCTION IN RHODOPHYTA: THE EXAMPLE OF GRACILARIA GRACILIS AND G. DURA SIBLING SPECIES

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In most populations of *Gracilaria*, the life history is of the *Polysiphonia* type, i.e. having morphologically identical diploid tetrasporophyte and haploid gametophyte phases, the latter consisting of equal numbers of male and female plants. The female gamete is fertilized *in situ* and the zygote develops as a third phase, the diploid carposporophyte, a spore-producing structure entirely dependent on the female gametophyte. The resulting carpospores, the product of a single fusion of gametes, are numerous and genetically identical. Each spore can develop into a diploid tetrasporophyte in which reproduction involves meiosis, resulting in haploid genetically variable tetraspores. From *in vitro* demonstrations one would expect only male and female gametophytic and tetrasporophytic individuals in natural populations but in many cases in nature this is not so, indicating variations to this clear-cut sequence. For example, in some cases small gametophytic thalli develop as epiphytes on parental tetrasporophytes or in other cases male and/or female organs occur on the same thallus with or

without tetrasporangia. In this context, we have studied sex phenotypes in some populations of the *Gracilaria gracilis/Gracilaria dura* species complex. First results using population genetic approaches suggest that some taxa have abandoned classical sexual reproduction, reproducing asexually by suppressing or modifying meiosis and producing diploid spores that can develop without fertilization.

5OR.7

COCCOLITHOPHORE LIFE CYCLES: UNDERSTANDING PHYSIOLOGICAL AND ECOLOGICAL DIFFERENCES BETWEEN LIFE CYCLE PHASES

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Coccolithophores are most recognizable for their production of intricate calcium carbonate platelets, called ‘coccoliths’, which lead them to play an important role in the carbon cycle. The production of coccoliths is however not their only unique trait. As haplo-diplo-tonic organisms, coccolithophores are able to reproduce asexually in two different life phases (the ‘haploid’ and ‘diploid’ phase). This is in contrast to most phytoplankton, which can reproduce asexually in only the haploid or diploid phase. The haploid and diploid phases of the same coccolithophore species can differ significantly in size, calcification status, and abundance. The two cell forms may furthermore inhabit different niches, which has proposed to provide a competitive advantage in variable ecosystems. To date research has however primarily focused on the diploid life phases, and relatively little is known in regards to the biogeography of the haploid life phase, and how this correlates to the biogeography of diploid life phases. Recent research suggests that haploids are preferably found in shallower, oligotrophic waters relative to diploid life phases of the same species, and that haploid distribution is highly seasonal. Through a synthesis of coccolithophore abundance data and literature review, we have investigated these trends further and identified exceptions and key gaps in our knowledge. The findings will pave the way for further studies examining the physiology and ecology of coccolithophore life phases using both laboratory and ecological modeling approaches.

5OR.8

HIGH CELL DENSITY AND VIRAL INFECTION TRIGGER FORMATION OF RESTING STAGES IN THE MARINE DIATOM *CHAETOCEROS SOCIALIS*

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Many diatoms have heteromorphic life cycles that include the formation of resting stages. These stages can increase the range of conditions in which species can survive, thus ensuring their persistence in time with important ecological implications for population dynamics. Although common to many species, cues and modality of resting stage formation remain unclear. Nitrogen starvation is reported as the most effective one in the laboratory; however, the link between spore formation and nitrogen depletion in the natural environment remains elusive. Using the widespread marine diatom *Chaetoceros socialis* as model species, we tested additional factors that may play a role in inducing spore formation. Even though nitrogen depletion was confirmed to be the most effective trigger in laboratory conditions, resting stages were formed also when cell density was high but nutrients were not limiting and when cultures were grown in culture medium conditioned by high cell density. *Chaetoceros socialis* can be infected by a small RNA virus and we also tested if the viral attack can induce a life cycle shift from vegetative cells to resting spores. Indeed, significantly higher numbers of spores were produced in infected cultures as compared to the non-infected control. Our results

suggest the presence of chemical cues that induce the formation of spores and also suggest that biological interactions, both between cells of the same species and between diatom cells and the virus, can play an important role in this process.

5OR.9

SEX IN *ASTERIONELLA FORMOSA*?

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Asterionella formosa is a widely-distributed and well-studied freshwater pennate diatom but, unlike many other diatoms, sexual stages have never been observed. Sexual reproduction is presumed to occur in nature in *A. formosa* because mean cell length does not decline monotonically over time in lakes. The enigmatic lack of observed sex in *A. formosa* famously led David Mann to write a chapter in a Festschrift for J.W.G. Lund with the title 'Why didn't Lund see sex in *Asterionella*?' We grew clones of *A. formosa*, isolated from one of the lakes in the English Lake District that Lund studied, Esthwaite Water, under different conditions of light, temperature and medium composition. Using light- and epifluorescence microscopy and videos we observed what appears to be auxosporulation in *A. formosa*. Isogametes were produced and fused leading to the formation of auxospores with biovolumes equivalent to that of a full-sized cell. Auxospores were observed in mixed clones but also in monoclonal cultures. These observations of sexual reproduction help to explain how cell length is maintained in nature. The lack of observed gametes and auxospores in open water samples might be because they were not recognized or were destroyed by the fixation procedure, but alternatively sexual reproduction might not occur in the pelagic zone.

6. Biofilms and microphytobenthos: community composition, physiology, functional aspects, interactions with bacteria

6KN.1

A STICKY SITUATION: UNDERSTANDING THE MOLECULAR MECHANISM OF DIATOM UNDERWATER ADHESION

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Diatoms are unicellular microalgae that together with bacteria dominate marine biofilms in sunlit marine environments. The underwater adhesion of diatoms is accomplished through the secretion of carbohydrate-rich extracellular polymeric substances (EPS). In motile pennate diatoms, the adhesive EPS are secreted through a specialized slit in the cell wall (termed raphe) and are deposited as trails on the substratum thus providing the traction required for cell motility. So far, the molecular composition of the diatom adhesive trails has remained poorly characterized. Recently, we have developed a method for isolating cell-free diatom adhesive trails and demonstrated that they contain a complex mixture of carbohydrates and proteins. Unlike the well-characterized adhesion mechanism of marine mussels, diatom adhesion does not appear to be mediated by proteins contains dihydroxyphenylalanine (Dopa). To gain a deeper understanding of the role of proteins in diatom adhesion we have performed proteomics analysis of the adhesive trails isolated from the fouling diatom *Craspedostauros australis*. A number of novel proteins were identified that contain sequence features that are reminiscent of those found in extracellular and bacterial cell-wall anchored proteins (Choice-of-Anchored A domain, von Willebrand factor type A domain, PTS-rich). GFP-tagging and immunolocalization studies confirmed the presence of some of the identified proteins within the adhesive trails and biofilms. This work provides insight into the biomacromolecular structure of diatom EPS trails and lays the foundation for unraveling the molecular mechanism for diatom underwater adhesion.

6KN.2

NEAR-INFRARED LIGHT DRIVEN OXYGENIC PHOTOSYNTHESIS IN BIOFILMS - NOVEL INSIGHT TO THE ECOLOGY OF CYANOBACTERIA WITH FAR-RED ABSORBING CHLOROPHYLLS

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Far-red absorbing chlorophylls are constitutively present as Chl d in the cyanobacterium *Acaryochloris marina*, or dynamically expressed by the synthesis of Chl f and red-shifted phycobilins via far-red light photoacclimation in a range of cyanobacteria, which enables the use of near-infrared radiation (NIR) for oxygenic photosynthesis. While the biochemistry and molecular physiology of cyanobacteria with far-red chlorophylls has been unraveled in culture studies, the actual habitats and ecological significance of such cyanobacteria remain largely unexplored as no data on their in situ activity exist. However, an increasing amount of reports demonstrate that cyanobacteria with far-red absorbing chlorophylls are linked to shaded biofilm habitats. With a novel combination of hyperspectral imaging, confocal laser scanning microscopy, and nanoparticle-based O₂ imaging, we have demonstrated substantial NIR-driven oxygenic photosynthesis within a natural beachrock biofilm habitat by endolithic, Chl f-containing cyanobacteria. In this keynote, I present a detailed account of the spatial organization and in situ activity of Chl d and f-containing cyanobacteria in a widespread ecological niche, which indicates an important role of NIR-driven oxygenic phototrophs in endolithic biofilm habitats.

6OR.1

INTERACTIONS BETWEEN DIATOMS AND BACTERIA IN FRESHWATER PHOTOAUTOTROPHIC BIOFILMS

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Epilithic photoautotrophic biofilms represent complex communities of algae and bacteria, allowing cooperative nutrient utilization, production of extracellular polymeric substances, and intercellular communication. The freshwater biofilm-forming diatom *Achnantheidium minutissimum* and its naturally associated bacteria were isolated from biofilms of Lake Constance. In the presence of its associated bacteria, the xenic diatom produces insoluble carbohydrates forming stalks and capsules, which facilitate attachment to surfaces. After removal of bacteria, axenified diatoms no longer form biofilms and grow in a suspended mode, not adhering to surfaces. The diatom satellite bacterium *Bacteroidetes* sp. 32 was isolated from the same location as *A. minutissimum*. This bacterium is known to induce the stalks, capsules, and thus biofilm formation by excreting unknown bioactive molecules, while the bacterium can feed on the carbohydrates excreted by the diatom. This transition to a benthic lifestyle is evidently accompanied by changes to the photophysiology of the diatom, typified by changes in pigment concentrations and electron transport rates. This tight interdependence of this diatom and the bacteria in biofilms also raises questions about the benefits of such communities with regard to responses to environmental changes. For this purpose, we have studied the generation of such mixed-species photoautotrophic biofilms under controlled conditions in a sterile incubator system for biofilms. Taken together, our study demonstrates the impact of nutrient limitation and associated physiological acclimation of diatoms during biofilm formation.

6OR.2

THE WAY YOU MOVE: THE VERTICAL MIGRATORY RHYTHM OF INTERTIDAL MICROPHYTOBENTHOS IN SEDIMENT DEPENDS ON THE PHOTOPERIOD, THE LIGHT INTENSITY AND SPECTRUM

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Estuarine intertidal flats strong productivity is mainly based on the biological activity of benthic microalgae communities, or microphytobenthos (MPB), mostly dominated by diatoms. Epipelon is a major MPB growth form comprising motile species which undergo vertical so-called 'migration' patterns in the upper sediment layers. Typically, at the beginning of daylight emersion (but not during nighttime), epipellic diatoms move upward and form dense and transient photosynthetic biofilms at the surface of the sediment, while usually before and/or when immersion and/or night start, they move downward in the deeper sediment layers. While this well-known behavior has been extensively studied for more than a century, it remains an enigma. Indeed, this unique motility pattern repeats according to tidal and diurnal cycles and periods, continuously resetting to fit the everyday tides and the seasonal changes in day length. Because of such pattern, it is believed that epipelon migratory rhythms are driven by a mix of endogenous internal clock of unknown nature, and by diverse environmental stimuli, the later probably entraining/resetting at least part of the clock system. Among the environmental stimuli impacting on epipelon vertical migration, light is probably the most important. The present work intended to further decipher its role. Rhythmic changes in surficial abundance of natural MPB assemblages were continuously assessed by Imaging-PAM fluorimetry over several days in sediment sampled at different seasons (85 migration profiles from 40 sediment samplings over 2 years), and exposed to differential light conditions. In particular, we manipulated i) the natural photoperiod MPB was acclimated to in order to disentangle the tight link between the diurnal and tidal rhythmicity of epipelon migration, and ii) the light spectrum in order to potentially impact on MPB accumulation at the surface of sediment: we found a specific and positive effect of blue wavelengths that was diatom species-dependent.

6OR.3

THE ECOLOGICAL CONSEQUENCES OF SPECIFICITY IN DIATOM BACTERIA ASSOCIATIONS

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Benthic diatoms and heterotrophic bacteria live in close association in marine intertidal mudflats. While it has been shown that diatom species tend to differ in their associated bacterial taxa, the implications of this specificity for the algal host and more broadly, the functioning of microphytobenthos, are currently not known. To test if this specificity results in the improved fitness of the host, we exposed diatoms to microbiomes initiated from the same natural bacterial community but selected by different hosts. The assembly process was accompanied by a reduction in competition between host and bacteria and indeed led to species-specific algal microbiomes. We further show that when these microbiomes were confronted with another diatom host, competition increased as a function of phylogenetic distance to the original host diatom species. By challenging a synthetic diatom community, consisting of *Cylindrotheca closterium*, *Navicula phyllepta* and *Seminavis robusta*, with a natural bacterial inoculum, we verified if these species-specific effects on diatom fitness would also have implications for the outcome of competitive interactions between diatom species. The bacteria differentially impacted the growth of the different diatom species, resulting in compositional shifts in this community. Together, our results suggest that the specific interactions between bacteria and diatoms are a key factor in determining community composition and growth of benthic diatoms in intertidal flats.

6OR.4

PHYTOBENTHIC BLOOM DYNAMICS WITHIN A TROPICAL MESOCOSM SEAGRASS COMMUNITY

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Eutrophication is one of the main drivers affecting phyto-benthic communities in coastal habitats. Tropical seagrass ecosystems are particularly vulnerable to increasing nutrient pollution and seagrass loss can be exacerbated under global warming scenarios, as levels exceed temperature tolerance limits, providing a competitive advantage to different phyto-benthic bloomers. To examine the phyto-benthic community dynamics and study their responses to nutrients and temperature alterations, a multifactorial mesocosm experiment mimicking an Indo-pacific seagrass community was set up. The single and potential synergistic effect of nutrients (low and high doses of N+P) and temperature (25° and 31°C) were tested on the composition and physiology of phyto-benthic algae and biofilms. Changes in the phyto-benthic community pattern were followed by sequencing photographs and micro phyto-benthic compositions were confirmed by microscopic and molecular investigations. Preliminary data showed, bloom formation of different phyto-benthic taxa along the benthic algal succession trajectory. As blooms of dinophyceae (e.g. *Prorocentrum* cf. *lima*) and chrysophyceae peaked over a rather short time, the abundances of the cyanobacterial Oscillatoriales (e.g. *Oscillatoria* spp.) seem to be linked to the trajectory of either a green (*Cladophoropsis* sp.) or red macroalgal (*Laurencia* sp.)-dominated state within the nutrient-enriched treatments. In contrast, the brown tide forming pelagophyceae (e.g. *Aureococcus* sp.) dominated the phyto-benthic communities under nutrient-depleted conditions. Phyto-benthic alterations were also reflected in photosynthetic performances (e.g. measured as rETR and biological oxygen demand, measured in sediment and water column).

6OR.5

COUPLING PAM FLUOROMETRY, C-FLUXES AND REFLECTANCE TO ESTIMATE MICROPHYTOBENTHOS PRIMARY PRODUCTION BY HYPERSPECTRAL REMOTE-SENSING

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Tidal flats are amongst the most productive marine ecosystems on the Earth mainly due to the presence of microscopic algae colonizing sediments (i.e. microphytobenthos). These ecosystems provide important services such as biodiversity depositories, storm protection, shoreline stabilization, food production and contribute to the Blue Carbon. They are one of the most extensive coastal ecosystems, with an estimated global area of ~130.000 km². With a global annual Gross Primary Production (GPP) estimated to be in the order of 500 million tons of carbon, these ecosystems can be responsible for up to 20% of the ocean GPP although occupying only 0.03% of their surface. In spite of their potentially high contribution to the Global Carbon Budget, their actual contribution remains unknown. The only way to fill this gap is the use of remote sensing imagery calibrated with C-flux measurements, especially with the use of hyperspectral technology. With several dozens of narrow spectral bands, this technology is the only one able to detect and map changes in relation to biological and physiological processes involved in GPP. The objective of this study is to couple hyperspectral reflectance (400-900 nm), PAM-fluorometry and C-fluxes measured on sediment cores colonized by natural biofilms under a range of light (50 to 2300 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and temperature (15, 25 and 40 °C) conditions. First, light curves from C-fluxes, expressed in $\text{mgC m}^{-2} \text{h}^{-1}$, and from PAM fluorometry, expressed in rETR (Electron Transfer Rate) and photosynthetic parameters (alpha, Ek and Pmax) for each temperature were compared. Second, reflectance data were used to retrieve the absorption cross-section (a^*) using the biofilm optical model (i.e the MPBOM). Third, radiometric indices, as the MBPLUE, were tested to estimate primary production expressed in rETR and $\text{mgC m}^{-2} \text{h}^{-1}$. Finally, GPP at the cores scale was mapped using hyperspectral reflectance in the function of light and temperature.

6OR.6

METAGENOMIC CHARACTERIZATION OF THE SURFACE BIOFILM ON MEDITERRANEAN LOGGERHEAD SEA TURTLES

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The external surfaces of marine vertebrates provide suitable hard substrata for the growth of rich microbial biofilms. Many aspects of turtle ecology and behavior are still unknown and the micro-epibionts may be used as an indicator of the animal behavioral pattern or as a proxy of the anthropogenic impact to coastal marine habitats of this species. However, the composition of these surface micro-communities is largely unknown. In recent years, numerous studies apply DNA metabarcoding approach using 16S and 18S amplicons to analyze different types of communities in various environments such as the surface layer of the ocean, sediments, and animal biofilms. In our study, we analyzed carapace and skin biofilm samples of ten loggerhead turtles (*Carretta carretta*) collected from distinct habitats in the Mediterranean Sea, along the Croatian and Italian coast of the Adriatic Sea and Amvraikos Gulf in Greece. The composition of OTUs representing bacterial communities living on the carapace and the skin were highly similar among both the Greek and Adriatic turtles reflecting taxa that are most abundant in the marine ecosystems such as proteobacteria (alpha, beta, gamma and delta), cyanobacteria, actinobacteria and bacteroidetes. The results of 18S amplicon analysis showed more variation between the Greek and Adriatic turtles, including different ratios of taxa which occupied the skin and the carapace. Eukaryotic OTUs that mostly dominated across samples were those of green, brown and red algae, crustaceans, ciliates and diatoms. This study provides valuable insights into how microbial taxa associated with animal surface biofilm vary among surface types and habitats which loggerheads utilize in the Mediterranean Sea.

7. Algae in a changing environment: perception of environmental signals, physiological changes, changes in community composition

7KN.1

STATISTIC MODELING OF AQUATIC PRIMARY PRODUCTION AND WATER MONITORING

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Different phytoplankton groups acclimate to certain growth conditions with respect to their physiological features. Mechanisms to cope with high light, alternative electron pathways and biomass composition are of special interest. These physiological features, however, alter the metabolic fluxes within the cell (carbon allocation) as well as the energy partitioning (usage of absorbed energy within different pathways). To analyze the quantum efficiency of each subprocess a variety of methods have been established: e.g. O₂/CO₂ gas exchange, chlorophyll fluorescence measurements and Fourier transformed infra-red spectroscopy. Our setup is used to develop an overall view of energy fluxes within phytoplankton cells with respect to abiotic factors (light climate, nutrient concentration, temperature, pH). Our recent results showed high plasticity, but also species-specific responses. These will be used for the interpretation of different growth strategies depending on the abiotic factors and therefore to model aquatic primary production as well as to optimize growth conditions in biotechnological applications.

7KN.2

PERFORMANCE OF POLAR MACROALGAE IN A CHANGING ENVIRONMENT: A SEASONAL PERSPECTIVE AND IMPLICATIONS FOR THE FUTURE

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The Polar ecosystems are among those more threatened by the changing climate. The increase in temperature in the Arctic is being particularly alarming. Hardbottom polar coastal areas are densely populated by macroalgae, many of which are present even during the long winter period. A number of campaigns have been carried out in Kongsfjorden (Svalbard), and common macroalgae species have been tested for their ability to perform in an increased atmospheric CO₂ and/or increased temperature scenario. We have shown that increased CO₂ can modify the carbon balance of different species in different ways, affecting both their ability to cope with summer excess light and their internal composition, in a manner different to their cold-temperate ecotypes (North Atlantic). But the new weather conditions are also threatening their ability to overcome long dark winter periods, compromising the interannual survival. An increase in winter temperature of 4 °C doubles the speed of thallus degradation and suppress the ability to generate new photosynthetic tissue. Taking into account that in western Svalbard the increase in winter temperature is about 3.6 °C per decade, we might be close to see a drastic change in the community. Primary production has been measured by three different methods (O₂ evolution, ¹⁴C fixation and PAM fluorometry), so uncoupling of photosynthetic processes have been revealed as part of the acclimation mechanisms. These adjustments allowed for the internal composition (carbohydrates, lipids and proteins) and growth being more resilient to the changing environment, so that acclimation is mainly taking part in the photosynthetic machinery. Photoinhibitory conditions occurring during the 24 h illumination during summer may explain some seasonal traits in these species.

7OR.1

EFFECTS OF POLLUTION ON POPULATIONS OF *CYTOSEIRA CRINITA*

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In the Mediterranean Sea, species of the genus *Cystoseira* (Order Fucales) are amongst the most important canopy-forming seaweeds on photophilic rocky bottoms. They provide high stand structure, biomass, food and habitat for many other associated organisms creating very productive, complex and diverse assemblages. Nevertheless, *Cystoseira* species are highly vulnerable to anthropogenic disturbances. *Cystoseira crinita* is a Mediterranean endemic species that develops a complex assemblage in shallow rocky habitats. During the last decades, *C. crinita* is facing severe declines and even local extinctions in different areas from Spain, France, Italy, Croatia, Montenegro and Greek coasts being extremely vulnerable. Some of these losses have been direct consequences of habitat destruction and overgrazing by sea-urchins, but frequently have been attributed to sea surface pollution and eutrophication. However, up to now, there is no experimental evidence of the impacts of pollution on *C. crinita* populations. Therefore, this study aims to evaluate for the first time the impacts that real conditions of pollution can cause to populations of *C. crinita* through laboratory experiments. Different sources of common pollutants such as nitrates, heavy metals (copper and lead), and herbicides (glyphosate) were applied to adults and juveniles of *C. crinita* during 6 months. Early and adult stages were monitored to determine the possible effects of pollutants at the biological and physiological level. For adult specimens, survival, growth, fertility level and photosynthetic activity were monitored once a month. For early stages, density and size were measured in months 3 and 6. After 6 months of treatment, adult specimens were tolerant to moderate levels of pollution. However, the survival and growth of early stages were compromised already after 3 months of treatment being glyphosate and nitrates the pollutants with the most severe effects. This higher sensitivity of early stages in front of pollutants could compromise the long-term viability of these populations. Ascertain the effects of current levels of pollution in both adults and early stages and identify the most harmful contaminant will help to get a full understanding of the biological and ecological consequences and can contribute to develop effective conservation plans and recovery strategies.

7OR.2

CRUSTOSE CORALLINE ALGAE AND OCEAN ACIDIFICATION: THE ROLE OF EVOLUTIONARY HISTORY

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Crustose coralline algae (CCA) are fundamental to the functioning of tropical and temperate marine ecosystems. In coral reefs, CCA are important for reef cementation, substrate stabilization and reef resilience. However, CCA are sensitive to the impacts of global climate change. Our knowledge of the effects of ocean acidification and warming on CCA has increased substantially in the last 10 years, however, little is known about the variability in responses among coralline algal species to climate change, and particularly on the role of evolutionary history as drivers of the CCA responses. To fill in this knowledge gap, we tested the effects of variable pCO₂ and temperature scenarios on a range of CCA species from the Great Barrier Reef, Australia. The selection of species included representatives of different evolutionary lineages, including basal (Sporolithales) and more derived groups [Hapalidiaceae, Lithophylloideae, and Mastophoroideae]. We assessed a range of physiological (photosynthetic oxygen evolution, maximum quantum yield, carbon stable isotope values), mineralogical (mineral composition) and individual (growth, calcification, survival) responses across species to gain insights into potential processes and mechanisms explaining the observed variability among lineages. We found considerable variability in the metrics across species, but there are trends of declining growth with increased pCO₂. Basal taxa seem more robust to the impacts of ocean acidification than those from the more derived groups. We also found some flexibility in the carbon uptake mechanisms in response to elevated CO₂ and the strength on this response varied across taxa. Our study provides insights into the biological traits that may have facilitated the survival and success of some CCA to past ocean acidification and warming events. This information is important to predict taxa that may be more sensitive to rapid climate change, and understand ecosystem functions that are at risk due to increased CO₂ emissions to the atmosphere.

7OR.3

DRIVERS OF THERMAL TOLERANCE OF A COSMOPOLITAN KELP SPECIES: HABITAT SUITABILITY, POPULATION QUALITY OR LOCAL ADAPTATION?

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The giant kelp *Macrocystis pyrifera* is a cosmopolitan species of cold-temperate coasts, yet encompassing a considerable temperature gradient across its distribution range, from 3 to 20°C. Its large distribution may strongly rely on a great dispersal capacity to (re)colonize distant habitats and maintain the species cohesion through gene flow. Yet, dispersal is expected to counterbalance diversifying selection on thermal tolerance. This study aimed at testing for the existence of local adaptation among regions separated by up to 4000km and strong thermal divergence. Common garden experiments mimicking reciprocal transplants were performed, each habitat being assimilated by a given temperature corresponding to the regional average of the sampled populations. Several proxies of fitness were measured in the haploid stage of the kelp, and sympatric versus allopatric conditions were compared. Additionally, a heat wave at 24°C was applied to measure the tolerance limits of these gametophytes. Significant differences among regions were observed in the responses to thermal variability, yet local adaptation *sensu stricto* was not detected. Fertility was more influenced by the geographic origin than by temperature *per se*, with possible effects of marginal conditions at the extremes of the distribution range. The effect of deme quality was also strongly determinant, suggesting that local stochasticity (i.e. genetic drift) may actually restrict the process of local adaptation. Small effective population sizes and low gene flow were confirmed by a population genetic survey, which also revealed a slight correlation between genetic diversity and average fitness of the populations. Even though all the gametophytes were able to survive at 24°C, a temperature rarely met at the northern limit of the distribution range (i.e. during major heat waves), the capacity to evolve adaptations to local or regional temperature regimes seems hampered by the effects of local stochasticity.

7OR.4

THE BOOM-BUST INVASION BY *LOPHOCLADIA LALLEMANDII* HAS A LOWER IMPACT THAN EXPECTED ON NATIVE ECOSYSTEMS

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Invasions of marine habitats by exotic algae are of major ecological concern since long-lasting negative effects have been reported on the diversity and the functioning of invaded ecosystems. Almost all studies on the impacts of invasive algae rely on observational comparisons of native diversity in invaded and un-invaded locations only a few years after the start of the invasion. However, many times, the invasion shows a boom-bust dynamics, with a progression from a transient “harmful” stage to a more persistent “harmless” phase. Here we show a first example on the effects of an invasive alga with a boom and bust dynamics on different native communities during a medium-term (15 years) invasion process. We monitored *Lophocladia lallemandii* abundance and native species composition from its introduction to 15 years later in two different MPAs. Initially, *Lophocladia lallemandii* exponentially increased in abundance, reaching coverage values > 70% in only 5 years; however, after some years when coverage values were maintained, *Lophocladia* steeply declined to much lower abundances (<15%). Changes in native species composition were detected during the boom phase, but native communities tended to return to its original state in the bust phase, showing a great capacity for recovery. We conclude that the final impacts of *Lophocladia* on native communities will strongly depend on whether this bust phase remains or it returns to a boom phase. Our results question the long-lasting effects of macroalgae invasions on native species, which is of critical importance for predicting ecosystem change and for managing the invasion.

7OR.5

CAN THERMAL HISTORY SHAPE THE THERMOTOLERANCE RESPONSE OF MEDITERRANEAN MARINE HABITAT-FORMING MACROALGAE?

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Macroalgae of the genus *Cystoseira* (Order Fucales), are the main marine canopy-forming species on photophilic Mediterranean rocky bottoms. During the last decades, several populations from different areas of the Mediterranean Sea have gone missing mainly due to habitat destruction, pollution and overgrazing although no evidence of warming impact has yet been published. After observing for the first time a negative impact of high temperatures on a *Cystoseira crinita* population, we experimentally tested how thermal stress affects the growth, ecophysiology and survival of this species. We aimed to acquire basic information about the thermotolerance features of *C. crinita* and to evaluate the role of history acclimation (particular thermal regimes) on thermal stress responses. To do so, we compared the thermal tolerance of populations dwelling in the coldest and warmest areas of the Mediterranean Sea by means of aquaria cultures at different temperature treatments (from 26°C up to 29 °C). We show that *C. crinita* populations from warmer areas like those thriving in the Eastern Mediterranean showed a temperature tolerance threshold 2°C higher than those living in colder areas. Moreover, we demonstrate a strong correlation between the observed differential responses and the local temperature regimes experienced by each population. These results are one of the first evidence for the role of thermal history in shaping the thermotolerance responses of habitat-forming macroalgae under contrasting temperature environments.

7OR.6

ECOPHYSIOLOGICAL RESPONSES OF SIX INTERTIDAL SEAWEEDS THAT FOLLOWED CONTRASTING ABUNDANCE SHIFTS IN THE LAST DECADES UNDER THE INFLUENCE OF MULTIPLE STRESSORS

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Temperature and nutrients are major drivers of the distribution of seaweeds. Recent local losses of seaweeds have been linked to climatic stressors like temperature or changes in upwelling intensity. A better assessment of these field observations requires disentangling the potential interaction between warming and nutrient supply on the ecophysiological performance of seaweeds. Using a factorial design, we investigated the effects of temperature and nutrient on growth, photosynthesis and respiration of six intertidal seaweeds that recently followed contrasting upward and downward abundance shifts in NW Iberia. For six weeks, seaweeds were exposed to conditions that simulated present (14 and 18 °C) and warmed (22 and 26 °C) thermal scenarios, combined with nutrient concentrations that emulated nutrient supply with (high) and without (low) the influence of the upwelling system. Nutrient supply modulated the effects of warming on the growth rate of some seaweeds only. Growth markedly slowed down in a warmed scenario in two of the downward group (*Mastocarpus stellatus* and *Fucus vesiculosus*), but only if nutrients were available in large quantity. Otherwise, growth was largely insensitive to temperature in the third representative of the downward group (*Chondrus crispus*) as well as in the upward group, regardless of nutrient supply. The temperature dependence of photosynthesis, showed consistent differences between trend groups. Regardless of the nutrient regimen, photosynthesis was insensitive to temperature in the upward group while water warming accelerated the photosynthetic rate in the downward group when nutrients were in great supply. Altogether, our results suggest that physiology and performance are more sensitive to warming in downward seaweeds, but only if nutrients are available in large quantity. From the perspective of future

scenarios, the differential effects of water warming on upward and downward seaweeds might greatly disappear if nutrient decrease in the region because of a weakening of the upwelling system.

7OR.7

ENVIRONMENTAL FACTORS SHAPING RUBISCO AND CARBON CONCENTRATING MECHANISM CO-EVOLUTION ACROSS AQUATIC ORGANISMS

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Despite the unquestionable importance of the main CO₂-fixing enzyme in the biosphere, Rubisco, its natural catalytic diversity, especially in aquatic organisms, still remains poorly understood. Aquatic photosynthesis not only forms the basis of marine and freshwater food webs, representing half of the global primary productivity but also exerts an important control on the global carbon cycle through regulation of the ocean's biological pump. Although Rubisco kinetics is currently an area of active study, there is also a lack of an integrative and complete meta-analysis of all the Rubisco catalytic data even reported. This analysis becomes an essential requisite to succeed in providing new relevant information on Rubisco phylogenetic evolution and environmental adaptation in aquatic ecosystems. With this aim, we undertook an exhaustive compilation of all the data published so far on the *in vitro* Rubisco catalytic parameters of aquatic macro and microorganisms, from bacteria to plants. Original data were corrected using a novel formulation to eliminate study-to-study effects. Rubisco evolution in aquatic organisms was re-evaluated in the context of environmental temperature, CO₂ and O₂ concentrations, and in the appearance and strength of carbon concentrating mechanisms. The analysis of these data revealed evolutionary trends in Rubisco kinetics among the different phylogenetic groups. Importantly, this analysis questioned the canonical trade-off between Rubisco affinity for CO₂ and velocity of carboxylation previously described for Spermatophyta. The existence of contrasting relationships between the catalytic traits across aquatic organisms is indicative of catalytic

plasticity among and within the phylogenetically diverse groups and seems to be related to the diversity of carbon concentrating mechanisms and the thermal environment. This information will be especially useful for understanding the effects in the productivity and ecological success of the different aquatic organisms in future scenarios of Global Change.

7OR.8

THE ROLE OF VOLTAGE-GATED ION CHANNELS IN DIATOMS

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Calcium signaling is employed ubiquitously in eukaryotes, as well as bacteria, to mediate their response to a range of abiotic and biotic stimuli. Upon sensing a stimulus, Ca²⁺ channels located in cell-membranes open and allow Ca²⁺ to quickly enter the cytosol. These changes in Ca²⁺ are detected by diverse Ca²⁺ binding proteins, triggering a range of downstream responses. Within the Ca²⁺ signaling toolkit, voltage-gated channel calcium channels are known for their fast opening and closing kinetics, making them a vital part of rapid signaling events such as muscle contraction or neuronal action potentials in vertebrates. We are examining calcium signaling mechanisms in unicellular algae in order to better understand their cellular functions. In particular, we are interested in the role of voltage-gated calcium channels in marine diatoms. The genomes of *Thalassiosira pseudonana* and *Phaeodactylum tricorutum* possess a surprisingly sophisticated array of voltage-gated ion channels. Little is known in what context these algal voltage-gated Ca²⁺-channels are used, but their diversity suggests that different signaling mechanisms from those found in land plants or animals might be present. We are currently investigating the role of the voltage-gated Ca²⁺ channels in marine diatoms using a range of molecular and physiological techniques. These include live cell imaging to examine the sub-cellular dynamics of Ca²⁺ in response to a range of environmental stressors such as osmotic stress. In addition, gene knockout technologies are being employed to examine the roles of specific ion channels in these signaling processes. The results indicate

that diatoms possess unique mechanisms for sensing and responding to their environment and more widely provide insight into the evolution of calcium-dependent signaling processes in eukaryotes.

7OR.9

EFFECTS OF CHANGING PHOTOPERIOD ON THE REGULATION OF PHOTOPHYSIOLOGY IN THE POLAR DIATOM *FRAGILARIOPSIS CYLINDRUS*

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In the Arctic Ocean, the availability of light is mainly controlled by the seasonal dynamic of sea-ice and snow cover. The on-going impact of climate change results in an early melting of the snow-ice cover in spring leading to a general increase in the available light intensity. The light dose that is sufficient to allow algal growth is now reached earlier in spring thus extending the productive period. However, in these conditions, Arctic diatoms experience larger seasonal light dose changes due to the extreme natural photoperiod (i.e. from potentially complete darkness in winter to continuous light in summer). The impact of photoperiod on the regulation of photophysiological processes of arctic algae is largely unknown. The aim of this study was to characterize the phenological and rhythmic response of the cold-adapted diatom species *Fragilariopsis cylindrus* to representative seasonal photoperiods observed at 67° N. Cells were acclimated for three weeks in bioreactors with light:dark photoperiods of 0 h:24 h (middle/end of December), 6:18 (early February), 12:12 (end of March), 18:6 (early May) and 24:0 (end of May). Experiments were conducted at 0°C and with an illumination of 30 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, the optimal irradiance for growth. Our results suggest that some aspects of the photophysiology of *F. cylindrus*, especially photoprotection (non-photochemical quenching and xanthophyll pigments content), have strong photoperiodic rhythmicity. We further exposed

F. cylindrus from an alternance of light:dark photoperiod (18:6) to either complete darkness (0:24) or continuous light (24:0). Results suggest that the observed photophysiological rhythmicity might depend on an endogenous circadian regulation based on the regular alternation of light and dark phases. *F. cylindrus* shows extraordinary light-response abilities allowing it to grow under the extreme Arctic seasonal light conditions and transitions, mainly through a finely tuned photoprotection ability.

7OR.10

WHY IS THE PLASTID BLUEGREEN IN THE RED ALGA *CHROOTHECE MOBILIS* PASCHER & PETROVÁ (STYLONEMATOPHYCEAE, RHODOPHYTA)?

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Chrootheca mobilis is a red alga from salt-rich flooded terrains. It is seldom reported and always in very shallow water bodies or in marshes. A distinct character of this species is its bluish colour, turning into yellow-orange with high light intensity, that contrasts with the red colour typical of its phylum. Confocal Laser Microscopy (CLMS) showed the complexity of its 3-D stellate chloroplast and failed to detect phycoerythrin in any of the strains and species of the genus studied. For the examination of the underlying genetic causes of the particular colour of *Chrootheca mobilis* its plastid genome was sequenced using Illumina HiSeq technology. We assembled and annotated a draft plastid genome (186,153 bp) that contained 179 CDSs. In the plastid genome of *C. mobilis*, the gene *cpeB* that encodes for the β subunit of phycoerythrin was present but the gene *cpeA* gene that encodes for the α subunit was absent. These genes form a single operon in all other red algal plastid genomes that have been analyzed. This finding provides the molecular basis to understand the absence of phycoerythrin in this red algal genus and its particular colour.

7OR.11

GLOBAL WARMING AND OLIGOTROPHICATION SYNERGY LEADS TO GREATER PHYTOPLANKTON LIPID PRODUCTION

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Earth temperature is rising and oligotrophication is becoming apparent even in coastal seas. In a highly changing environment, phytoplankton use carbon and nutrients to form important biomolecules, including lipids. However, the link between lipid production and changing environment is still unexplored. In order to investigate phytoplankton lipid production under different temperature, salinity and nutrient regimes we conducted batch cultures and *in situ* experiments. The diatom *Chaetoceros pseudocurvisetus* cultures were grown under controlled temperatures ranging from 10 to 30 °C and nutrient regimes mimicking oligotrophic and eutrophic conditions. Results were compared to plankton community's lipid production in the northern Adriatic at two stations considered as oligotrophic and mesotrophic during an annual monthly sampling. In order to gain detailed information on the investigated system, we supplemented lipid data with chlorophyll *a* concentrations, phytoplankton taxonomy, cell abundances and nutrient concentration along with hydrographic parameters. We found enhanced particulate lipid production at higher temperatures, and substantially higher lipid production in oligotrophic conditions. Lipid remodeling, including change in the ratio of phospholipids and glycolipids, is more affected by the nutrient status, than the temperature increase. Triacylglycerol accumulation was observed under the nitrogen starvation.

7OR.12

GHOST OF THE PAST: EVIDENCE OF ECOLOGICAL MEMORY IN PHYTOPLANKTON COMMUNITIES EXPOSED TO LONG-TERM HERBICIDE APPLICATION

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Long-term exposure to stressors can induce physiological and evolutionary changes in individuals that tend to persist over multiple generations. This recollection of information from antecedent events that helps ecosystems to recover and prevent catastrophic failures from recurring stressors has been termed *ecological memory*. However, this concept still lacks validation. We addressed this knowledge gap by using a two-phase lab controlled experimental approach with the aim of differentiating between long-term adaptation vs short-term acclimation of natural phytoplankton communities originating from different historical contamination backgrounds. Sediments containing seedbanks were collected from two Swedish lakes: a near-pristine lake (Finnsjön) in a forested catchment and an impacted lake (Tåkern) located in an extensive agricultural catchment with long-term contamination by herbicides. During *phase I*, phytoplankton communities from the two lakes were germinated in the presence and absence of an herbicide isoproturon (12 µg/L). In phase 2, the communities from *phase I* was exposed to four concentrations of isoproturon (7, 12, 61, 92 µg/L). The biomass development, photosynthetic efficiency (maximum quantum yield of photosystem II) and community composition were tracked. The presence of the herbicide in *phase I* had negligible effects on germination. In *phase II*, the two highest exposure levels significantly reduced the total biomass production, with Tåkern generally performing better. However, the presence/absence of herbicide during the germination phase had contrasting consequences for the communities' performances in *phase II*. Herbicide exposure during germination had a stronger detrimental effect

on the recovery of photosynthetic yield and increased the dissimilarities in communities' structures for the two highest exposure levels in Finnsjön. In contrast, Tåkern became more resistant, which might be the result of selecting species/ecotypes from a genetic pool that are already accustomed to herbicides. These results show that positive effects of short-term acclimation only occurred when communities had previous repeated encounters with the stressor.

7OR.13

LONG-TERM PHYTOPLANKTON CHANGE IN A HEAVILY IMPACTED COASTAL AREA IN THE NORTH SEA BETWEEN THE 1970S AND 2000S

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In the last 50 years, the North Sea has undergone important environmental changes which have affected all trophic levels. Since the 1970s, both increases and decreases in phytoplankton biomass and production have been reported from different parts of the North Sea. These conflicting observations may be caused by methodological differences but may also reflect actual regional differences related to hydrodynamics, climate and riverine and Atlantic influence. We compared biomass, community structure and seasonality of diatom and dinoflagellate communities between the 1970s and 2000s, based on a newly compiled dataset from the Belgian part of the North Sea. This hydrodynamically and bathymetrically complex area is under strong human influence, and has been characterized by eutrophication (up to the 1980s) and de-eutrophication (1990s onwards), and pronounced long-term changes in turbidity and water temperature. Distinct changes were observed between the two periods. Diatoms showed a pronounced increase from late winter to summer, resulting in a more intense and extended growing season in the 2000s. Dinoflagellates increased year-round but especially in summer. The onset of both diatom and dinoflagellate blooms showed a marked shift towards an earlier bloom start. In addition,

while in the 1970s distinct seasonal community types were present, a striking seasonal homogenization had occurred by the 2000s. Finally, we observed a pronounced increase in harmful diatom and dinoflagellate genera. The observed changes are most likely due to an increase in sea surface temperature, water transparency and changing nutrient loads and ratios.

7OR.14

THE ICELANDIC MARINE ECOSYSTEM: MAPPING PHYTOPLANKTON IN A COMPLEX HYDROGRAPHIC ENVIRONMENT

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Iceland, located at a high latitude and economically reliant on local fish spawning grounds, is particularly vulnerable to climate change. The island is surrounded by four different water masses which create a very dynamic and complex hydrographic environment. Marine micro-organisms play a crucial role in the food web and geochemical cycles, yet many questions regarding the dynamics of phytoplankton in Icelandic waters remain. Novel molecular methods can contribute to existing knowledge by increasing sample throughput and a better characterization of small and morphologically similar taxa. The aim of the “Microbes in the Icelandic Marine Environment (MIME)” project is to give a better understanding of the underlying mechanisms that control the marine food web surrounding Iceland. Seawater samples were collected annually from 19 coastal and oceanic stations around Iceland in May 2011-2018 and seasonally for one year (August 2017, February 2018 and May 2018). At the stations, seawater was sampled at several fixed depths from surface to bottom (n = 348) and metadata were collected for each sampling event (temperature, salinity and nutrients). High-throughput amplicon sequencing of the May 2018 samples (n = 165) shows a considerably higher abundance and diversity of *Chlorophyta* than previously estimated for this region by microscopy. Preliminary data also show that certain areas around Iceland are dominated by different phytoplankton taxa, confirming the impact of contrasting water masses on phytoplankton diversity. Sequencing of the May 2012, August 2017 and February 2018

sample sets is in process, and the addition of these datasets could clarify these patterns. This presentation will show the diversity and relative abundance of the phytoplankton in waters around Iceland including taxa not previously identified in this region by traditional microscopic methods, such as *Mamiellophyceae* and different *Phaeocystis* species. Phytoplankton dynamics will be presented in the context of time and physical oceanography.

7OR.15

A BRIGHTER FUTURE? THE PHOTOPHYSIOLOGICAL RESPONSE OF BOTTOM-ICE MICROALGAL COMMUNITY DURING SPRING TO SUMMER TRANSITION IN THE ARCTIC

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The diatom-dominated microalgal community inhabiting the bottom centimeters of first-year sea-ice supports a large fraction of the annual primary productivity in the Arctic Ocean. During the most productive early spring to summer transition, sea-ice diatoms have to cope with a changing light environment due to a complex combination of increasing day length and solar irradiance, coupled with snow cover and ice melting. During the last decades, this ancestral seasonal scheme has been disturbed by the impact of climate change. As light transmittance through ice increases, it is foreseen that the average irradiance to which sea-ice diatoms will be exposed will increase, and subsequently the future pan-Arctic Ocean primary productivity. Nevertheless, the Arctic diatom physiological and metabolic bases for such prediction remains unclear: this is mainly due to our poor assessment of their photophysiology. In order to fill this gap, we have monitored the photosynthetic activity of bottom-ice microalgal community over an unprecedented long period from early May to mid-July during the Green Edge international field campaign. We paralleled our

monitoring with punctual light stress experiments during the ice microalgal bloom period. In addition, we performed similar experiments on two typical sea-ice diatom strains, *Nitzschia frigida* found to be dominant at our study site, and *Fragilariopsis cylindrus* whose genome has been recently sequenced. Our results show that the bottom-ice microalgal community is able to cope with changing irradiance conditions including a sudden light increase of relatively high irradiance. Its response appears well supported by strong photoprotective regulation, including a combination of processes such as non-photochemical quenching-NPQ, damaged-Photosystem II repair and modulation of the Rubisco content. Under alike experimental conditions, the response of *N. frigida* and *F. cylindrus* was generally similar to the one of the community, paving the path for further ecophysiological investigations at the finest molecular level under lab-controlled conditions.

7OR.16

TALES FROM A MELTING ARCTIC: MICROALGAL BLOOM PHENOLOGY IN AN ERA OF CLIMATE CHANGE

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Climate change is impacting the Arctic most severely already today and leads to profound changes in the physical and chemical environment with complex (and partly contradicting) implications for ecosystems. Ice (and snow) cover does not only control light availability but functions also as a barrier against wind-induced deep mixing. Melting will increase light availability, but also stratification which controls nutrient availability. In order to improve our projections of future Arctic productivity, we have studied intensively how algal bloom phenology in Arctic sea ice and water is controlled by its environment. In the FAABulous project (Future Arctic Algae Blooms - and their role on the context of climate change) we combined extensive field studies in several fjord systems in Svalbard with experiments and modeling. Our results indicate that distinct physiological and ecological responses of different algae groups determine the climate change 'winners' and 'losers' - and need therefore to be considered correspondingly. Sea ice

algae appear less capable of handling high irradiances, and more vulnerable to ocean acidification than their pelagic counterparts. Shorter (and thinner) sea ice coverage affects the timing of blooms with cascading effects on higher trophic levels, while nutrient availability determines the overall biomass production and duration of

an algal bloom. We, therefore, conclude that ice algae will have competitive disadvantages in the transition from a light-limited to a nutrient-limited production regime in the Arctic, and alterations in species composition and bloom timing will potentially affect grazers and higher trophic level production.

8. Omics and genetic tools for algal model organisms_ understanding the biology of algae using metabolomic, proteomic, and genomic approaches

8KN.1

ULVA, A MODEL SYSTEM FOR GREEN SEAWEEDS

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Seaweeds evolved several times independently. The degree of commonality in the genetics underlying the transitions from simple unicellular to (relatively) complex macroscopic and mostly multicellular thalli, however, remains to be determined. Several whole genome sequences of brown and red seaweeds resulted in interesting insights in the biology of these respective organisms. So far, however, no whole genome sequence was available for a green seaweed. The publication of the *Ulva mutabilis* genome (~100 MB) is possibly a game changer. I will discuss observations resulting from comparative genomics, highlighting gene family expansions associated with multicellularity, bacterial to eukaryote lateral gene transfer and the endogenous production of plant hormones in green seaweed development. Last, I will give an update on the progress to develop a toolbox for functional genomics in green seaweeds (promotor screening, transformation protocols, genome editing and protoplast regeneration).

8KN.2

MOLECULAR MECHANISMS REGULATING SEXUAL REPRODUCTION AND MATING TYPE DETERMINATION IN THE DIATOM *PSEUDO-NITZSCHIA MULTISTRIATA*

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Pseudo-nitzschia is an important genus of marine diatoms responsible for blooms in coastal and oceanic waters. We selected *Pseudo-nitzschia multistriata* as a model system to explore the process of sexual reproduction, which in diatoms is linked to the formation of large-sized F1 cells within the cell size reduction/restitution cycle. *P. multistriata* is a heterothallic species in which sex is induced when cells of the opposite mating type (MT) get in contact. We sequenced the *P. multistriata* genome, defined its meiotic toolkit and elucidated the gene expression changes occurring in the opposite mating types when they are exchanging signals that will trigger sexual reproduction. To further refine our knowledge of this process, we are studying the gene expression changes occurring at later stages, when gametes and F1 cells appear. Although only ~20% of cells engage in meiosis, all cells arrest their growth during the sexual phase. Transcriptional changes involve genes related to photosynthesis and nutrient uptake, strongly down-regulated, and genes related to DNA repair and epigenetic modifications, up-regulated compared to control conditions. A transcriptomic-based approach comparing groups of strains of opposite mating type has also been instrumental in identifying a set of genes that are preferentially expressed in one or the other of the two mating types. These mating type-related genes play a role in defining the mating type and in governing cell behaviour during sexual reproduction. For one of these genes, we hypothesize a function in the pheromone system of the species, and the heterologous expression of its protein product is underway. Finally, we identified structural differences in the genomic region for another mating type-related gene and demonstrated that it acts as a sex determinant in *P. multistriata*.

8OR.1**V FOR SEX CHROMOSOME**

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Brown algae (Phaeophyceae) represent a unique group to study aspects of sexual reproduction due to the rich variation of life cycles, fertilization modes and sex determination systems found in this class. Particularly interesting are the aspects of the evolution of sex determination during the haploid life stage (UV sex chromosomes) and the various degrees of sexual dimorphism between gametes of closely related species, ranging from isogamy to oogamy. With the advent of -omics techniques we now have access to the whole genome sequences and transcriptomic data for males and females of several brown algal species, which allows us to investigate the genomic architecture and evolutionary history of the sex chromosomes across Phaeophyceae and correlate it with the different life history traits. In this study, we applied specific bioinformatic pipelines to identify and analyze the structural and evolutionary features of the male (V) sex chromosomes across two major brown algal orders, the Ectocarpales and the Laminariales. We used high-quality genomic, transcriptomic and genetic marker data to construct genetic maps, annotate genic regions and determine the sex-chromosomes containing the sex-determining regions (SDRs) in *S. japonica* and *D. herbacea*. Comparative genomic analysis with the model brown alga *Ectocarpus* suggests that a core set of sex-linked genes is conserved across all the investigated species, however, the sex-chromosome regions are highly dynamic with modifications occurring in a lineage-specific fashion.

8OR.2**AUREOCHROMES: THE BLUE BOX OF DIATOMS**

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Aureochromes are blue light-dependent transcription factors so far only found in Stramenopiles. They are unique in their inversed effector-sensor (bZIP-LOV) topology. Four paralogues of Aureochromes have been identified in the model diatom, *Phaeodactylum tricorutum*, named Aureo1a, 1b, 1c, and 2. We investigated the role and regulation of the Aureochromes by a combined molecular and physiological approach. Accordingly, *PtAureo1a* expression follows a light-independent circadian rhythm, while *PtAureo1b* expression is light-dependent. Expression of *PtAureo2* is time and light independent. *PtAureo1a* was suggested to be involved in high light photoacclimation in *P. tricorutum*. To verify this phenotype, we complemented *PtAureo1a* knockout lines generated via TALEN technique with its wild type *PtAureo1a* gene. We demonstrated a new methodological approach for complementing knockout strains in diatoms. Strengths and pitfalls of this method will be presented here. Furthermore, we created and investigated *PtAureo1a* overexpression lines. While Aureochrome knockout and overexpression had important consequences on the photoacclimation phenotype of *P. tricorutum*, it also influenced the expression of the other Aureochromes, pointing towards a tight regulatory feedback mechanism between Aureochromes. We are now just beginning to understand the detailed molecular mechanisms for light regulation via blue light receptors in diatoms.

8OR.3**HOW HAPTOPHYTE MICROALGAE MANAGE VITAMIN B₁₂ LIMITATION**

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Vitamin B₁₂ (cobalamin) participates to the control of phytoplankton development and community composition in oceans. B₁₂-dependency is common among microalgae and seems unrelated across lineages, with an estimate half of species requiring this co-factor for growth. Cobalamin auxotrophy is characterized by the presence of B₁₂-dependent methionine synthase, a key enzyme involved in methionine biosynthesis, and the lack of a B₁₂-independent isoform. Despite their important role in carbon and sulphur biogeochemistry, little is known about how haptophytes use cobalamin and cope with the effects of B₁₂ limitation in the environment. Therefore, deciphering B₁₂ dependency and associated metabolism of this taxa is relevant for elucidating primary production and nutrient cycling processes in the ocean. Here we report an evaluation of cobalamin auxotrophy among Haptophyta lineage based on publicly-available molecular data for 19 species. We found that none of the species encode the B₁₂-independent methionine synthase, suggesting ubiquitous B₁₂ auxotrophy in this phylum. Considering actual literature, Haptophyta would be the only microalgae phylum gathering exclusively cobalamin-dependent species. We then addressed the effect of different levels and dynamics of B₁₂ on the molecular physiology of the model haptophyte *Tisochrysis lutea* by coupling growth assays in batch and chemostat, cobalamin quantification and gene expression analyses. We focused on genes encoding either B₁₂-dependent enzymes, methionine cycle proteins or intracellular cobalamin transporters. Our results suggest that haptophytes may use different strategies to counterbalance cobalamin deprivation. They can uptake dissolved methionine from seawater. They finely and quickly up-regulate methionine cycle genes when B₁₂ becomes rare and down-regulate genes involved in B₁₂ transport to the mitochondrion, presumably facilitating B₁₂ allocation for methionine synthase activity. Taken together, these results point out the importance of cobalamin in haptophytes cellular processes and give new insights for understanding how B₁₂-producing bacteria could shape the dynamics of this important phytoplankton community.

SOR.4

DNA-FREE GENOME EDITING USING CRISPR/CAS9 IN THE DIATOM *PHAEODACTYLUM TRICORNUTUM*

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Development of targeted nucleases for precise genome editing, like TALEN and CRISPR/Cas9, has strongly enriched the available molecular toolbox for the model diatom *Phaeodactylum tricornutum*. Stable knockout can now be achieved, allowing for a more stringent characterization of target gene function compared to classical RNAi-mediated knockdown approaches. However, the nuclease-encoding genes are most commonly transformed into *P. tricornutum* via particle bombardment or electroporation, two methods that have several drawbacks: The exogenous DNA is stably integrated into the genome at random loci, resulting in a highly variable transgene expression among transformants and potentially unintended disruption of additional genes. Furthermore, the integrated nuclease is continuously expressed, increasing the risk for off-target effects. Therefore, we aimed to reduce nuclease exposure time to a minimum by establishing a protocol based on biolistic delivery of Cas9 and gRNA as a ribonucleoprotein (RNP) complex. For selection of positive transformants without co-delivery of a plasmid encoding for an antibiotic resistance gene, the use of two endogenous marker genes was established: Knockout of *PtApt* (adenine phosphoribosyltransferase) conferring resistance to 2-fluoroadenine and knockout of *PtUmps* (Uridine-5'-monophosphate synthase) resulting in resistance to 5-fluoroorotic acid as well as an uracil auxotrophy. We then validated this approach by generating double knockout strains by simultaneously using RNPs targeting one of these endogenous markers and *PtAureo1a*, a gene encoding for a blue-light dependent transcription factor. About 30% of the clones obtained were biallelic *PtAureo1a* knockout strains, which showed a decreased capacity for photoprotection via non-photochemical quenching, confirming the phenotype previously observed in *PtAureo1a* knockout strains generated via plasmid-based delivery of TALENs.

SOR.5

NEW INSIGHTS INTO ALGAL MANNITOL BIOSYNTHETIC PATHWAYS

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Photosynthetic Eukaryotes produce mannitol, sugar alcohol, for carbon storage and in response to environmental stress. In algae, mannitol biosynthesis is a two-step process: (1) fructose-6-phosphate is reduced into mannitol-1-phosphate (M1P) by a reversible M1P dehydrogenase (M1PDH); (2) a M1P phosphatase (M1Pase) hydrolyzes the phosphate group to produce mannitol. A recent analysis of algal genomic and transcriptomic resources showed that potential genes coding for M1PDHs and M1Pases are prevalent across algal lineages with different evolutionary history. Two distinct types of M1Pases are currently known, histidine phosphatases (His-M1Pase), and haloacid dehalogenase phosphatases (HAD-M1Pase). M1PDHs and M1Pases occur as separate standalone enzymes, and as fusion proteins containing one M1PDH module fused with one M1Pase module. Both standalone and fusion proteins co-exist in some algae. In this context, mannitol biosynthetic genes from selected algae were expressed heterologously to assess corresponding enzymatic activity and further biochemical characterization. Among green algae, *Micromonas pusilla* potential standalone HAD-M1Pase and M1PDH/HAD-M1Pase fusion confer mannitol production to *Escherichia coli*. Subsequent characterization of the purified recombinant fusion protein revealed interesting biochemical properties. Another green alga, *Pterosperma* sp., harbours a unique fusion formed by an M1PDH module and a His-M1Pase module, and this protein is functional in *E. coli*. The haptophyte *Emiliania huxleyi* exhibits one standalone HAD-M1Pase and one standalone His-M1Pase, and both are active in *E. coli*. Among brown algae, analysis of the *Ectocarpus* sp. and *Saccharina japonica* genomes revealed the presence of several genes coding for M1PDHs and HAD-M1Pases. Corresponding recombinant enzymes have been characterized, and *Ectocarpus* HAD-M1Pases exhibit different substrate specificity compared to *Saccharina* orthologs. To complete this overview, *M. pusilla* fusion protein was considered for mannitol production in the industrial biotechnology chassis *Synechococcus*. While production rates were low in engineered cyanobacteria, results obtained lay the ground for optimization of

photosynthetic production of mannitol in industrially relevant organisms.

8OR.6

TOXIN BIOSYNTHESIS IN *PRYMNESIUM PARVUM*: FROM STRUCTURE TO GENES

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The haptophyte *Prymnesium parvum* is known to cause massive fish kills during its blooms. The toxicity of *P. parvum* is attributed to its ability to produce prymnesins, a group of supersized ladder-frame polyether compounds. This group of toxins can be further divided into three different types (A-, B- and C-type), which present further diversity in terms of number and chemical structure of derivatives. In the present study, we used nine *P. parvum* strains representing all three types of prymnesins to elucidate the molecular mechanisms involved in prymnesin biosynthesis. Due to the polyketide nature of prymnesins, particular attention was paid to polyketide synthase genes (PKSs). The transcriptomes of all nine *P. parvum* strains were screened for the presence of PKS genes. A mean of 25 contigs containing modular type I PKS ketosynthase (KS) domains were found per strain, which were subsequently used to assess the evolutionary history of prymnesin production. The phylogenetic analysis of the KS domains showed that, compared to KS transcripts from other organisms, they form distinct clades as well as clades corresponding to each prymnesin type. In addition, a comparative transcriptomic analysis of all strains revealed the presence of candidate genes involved in the biosynthesis of specific prymnesin derivatives. The current study will contribute to a better understanding of the mechanisms of toxin production in *P. parvum* in order to develop efficient tools for monitoring purposes.

9. Algae in their ecosystems: novel approaches to study algal ecology

9KN.1

DNA METABARCODING DEMONSTRATES THE IMPORTANCE OF RHODOLITHS FOR MACROALGAL ECOLOGY AND BIODIVERSITY IN GULF OF MEXICO DEEP-WATER MESOPHOTIC HABITATS

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Understanding the ecology and biodiversity of deep-water communities is a major challenge. In the NW Gulf of Mexico, unique deep bank habitats associated with salt domes occur at ~50-90m on the continental shelf offshore Louisiana and Texas. In these mesophotic rubble habitats rhodoliths, accreted by calcified crustose coralline red algae, are the main hard substrata for the attachment of benthic macroalgae. Metabarcoding of environmental DNA using molecular markers (including 16S gene V4 region and *tufA* gene) for endolithic portions of rhodoliths has revealed hidden cryptic algal diversity including spores, propagules, and unsuspected life history stages (e.g., dinoflagellates, haptophytes, unicellular red algae). We explored cryo-SEM as a potentially more informative method than regular SEM to minimize artifacts of sample preparation in the study of endolithic cell inclusions which brought to light a suite of microalgal stages. We were able to differentiate floridean starch from cellular inclusions. Analyses of combined 16S V4 metabarcodes and 16S Sanger sequences of several macroalgal orders increased the established record of diversity in the region. Progress is underway to link the eukaryotic component of the rhodolith

holobiont (“total organism”) with its co-occurring prokaryotic component. Rhodoliths are marine biodiversity hotspots that may function as seedbanks, temporary reservoirs for life history stages of ecologically important eukaryotic microalgae, or as refugia for ecosystem resilience following environmental stress.

9KN.2

APPLICATION OF HIGH-THROUGHPUT METABARCODING IN LIMNOLOGY AND PALEOLIMNOLOGY TO CHARACTERIZE CHANGES IN MICRO-EUKARYOTIC DIVERSITY FACING ENVIRONMENTAL PRESSURES

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The application of high-throughput sequencing for the analysis of environmental DNA (eDNA) offers many new areas of inquiry in limnology and paleolimnology, allowing to study the spatial and temporal dynamics of biological assemblages and their responses to anthropogenic and climatic pressure. Here, we illustrate recent applications aiming at characterizing the diversity of planktonic micro-eukaryotes and their turn-over at various time-scales. A large diversity of taxa is considered from phytoplankton (chlorophyta, bacillariophyta, ...) to micro-heterotrophs (ciliophora, fungi, cercozoa, ...). Diverse metrics as richness, composition, community structure and patterns of co-occurrence are taken

into account to study the responses of these biological assemblages to local anthropogenic disturbances (eutrophication) or more global pressures (temperature conditions). Multi-scale temporal rearrangements occurring within micro-eukaryotic communities are studied from both “modern” and “ancient” DNA. Retro-observation (analysis of sediment archives) provides an opportunity to place recent changes in a broader temporal context and better decipher the relative importance of environmental drivers affecting planktonic communities. The potential and challenges associated with the study of eDNA to address critical research questions in lacustrine ecology are discussed.

9OR.1

UNVEILING THE HIDDEN DIVERSITY OF PHOTOSYNTHETIC AND MIXOTROPHIC PROTISTS IN A LARGE AND DEEP SUBALPINE LAKE USING A HIGH THROUGHPUT SEQUENCING APPROACH

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The structure and abundance of aquatic microbial communities, microalgae, protozoans and fungi contribute to characterize trophic webs and productivity. Nevertheless, in freshwater environments, the majority of the investigations were historically addressed towards the study of microalgae, either pelagic (“phytoplankton”) or periphytic. These two broad functional groups are composed of a wide variety of photosynthetic and mixotrophic organisms that show specific adaptations to different lake typologies and trophic status. Traditionally, in addition to the protist fraction, microalgae also include photosynthetic cyanobacteria. Based on high throughput sequencing approaches (HTS) (16S rDNA), previous studies carried out in Lake Garda and in the other large lakes south of the Alps allowed identifying a higher number of cyanobacterial taxa compared to those previously characterized by light microscopy. In this contribution, we will characterize and critically discuss the composition, alpha diversity, and seasonal dynamics of planktonic photosynthetic and mixotrophic protists through a two-year study carried out in Lake Garda using HTS analyses (18S rDNA). The planktonic community showed a higher diversity during late spring and summer, caused by a greater development of many algal groups (mostly Ochrophyta, Chlorophyceae, Dinophyceae). The main pattern of seasonal change was comparable to that obtained

using the traditional microscopy methods. Compared to traditional approaches, for most of the algal groups (e.g. Zygnemophyceae, Dinophyceae) HTS allowed to discover a wider number of taxa never detected until then in the large lakes south of the Alps. Nevertheless, while HTS approaches enable increasing the knowledge of protists diversity, their use in the evaluation of target groups is not free of difficulties due, among the others, to the short length of 18S rDNA sequences obtained with present technologies. Further, the presence of varying copy number of 18S rDNA still represents a source of uncertainty in assessing community structure and seasonal dynamics in protists.

9OR.2

SURVEY OF PHYTOPLANKTONIC ALLELOPATHIC INTERACTIONS

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The plethora of coexisting phytoplankton species utilizing the same finite number of nutrients in the same ecological niche contradicts the competitive exclusion principle which states that the number of coexisting species cannot exceed the number of the limiting resources (Gause, 1932). This violation of the principle, known as the “paradox of the plankton” (Hutchinson 1961) has been studied extensively. However, our knowledge of the mechanisms responsible for this paradox and to what extent each of them contribute to that is still limited. Amongst the many hypothesized mechanisms, phytoplankton’s biochemical interactions, have been examined only scarcely and few relevant chemicals have been identified. Those interactions, that can be either beneficial or inhibitory, are known as allelopathy. Hitherto, in phytoplankton allelopathy studies, attention has been paid to toxic species and usually, the studies are restricted to a few highly studied systems. In order to determine at what extent biochemical interactions affect phytoplankton communities, we established a systematic and representative survey of allelopathic interactions involving 18 species from 14 different taxonomic groups, including both toxic and non-toxic species. With these 59 co-culture

combinations, we aimed to determine the prevalence of and magnitude of allelopathy in natural phytoplankton assemblages. Allelopathy accounted for the majority of all observations amongst surveyed phytoplankton. This surprising finding suggests that phytoplankton utilizes exuded chemistry to a greater degree than previously hypothesized and that phytoplankton likely have the ability to influence community structure significantly by tailoring their chemical environment. We also found that some species exhibit global patterns of resistance or susceptibility to multiple competitor species surveyed or patterns of negative interference or stimulation towards competitor growth. This survey supports that allelopathy could be an important driver of phytoplankton species' diversity in the open ocean.

9OR.3

CONNECTING THE MOLECULAR SPECIES CONCEPT OF THE GENUS *FRAGILARIA* (BACILLARIOPHYTA) TO ECOLOGY

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After a recent examination of diversity within the genus *Fragilaria* (Bacillariophyta), connecting morphological and molecular data to produce harmonized species concepts for part of the genus (Kahlert et al., in press), we now need to revisit the ecological preferences of the curated taxa, including three new described species, to be able to use this knowledge for research and environmental assessment. We first tested whether the *Fragilaria* taxa in the curated and updated open-access barcoding library Diat.barcode (Rimet et al. 2016, 2018a) can be separated by a short part (312bp) of the rbcL gene at all, because routine DNA metabarcoding of environmental samples is

usually performed on short barcodes due to technical issues. We then matched the short barcodes of the different taxa(-groups) to three sets of environmental samples with available ecological data: from France, the Disunited Kingdom, and Sweden. This was done by 1) processing all runs with the bioinformatics pipeline DADA2, 2) merging all the amplicon sequence variants (ASVs) tables, 3) extracting the *Fragilaria* sequences, and finally 4) linking them to the environmental data. We assume that the better taxonomic discrimination obtained using DNA metabarcoding translates into better ecological understanding, or at least clearly shows the limits of using barcoding for environmental analysis and assessment.

9OR.4

ON THE SHOULDERS OF GIANTS: WHAT EPIZOIC DIATOMS ARE TEACHING US ABOUT DIATOM EVOLUTION

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Our knowledge and understanding of diatom diversity, diversification and evolution increase with every collection made from benthic marine habitats. In recent years, one such habitat has been the body surfaces of sea turtles and manatees. This habitat has yielded many new data, particularly on taxa historically associated with the Rhoicospheniaceae. For example, we have found both photosynthetic and non-photosynthetic species of the genus *Tursiocola* Holmes, Nagasawa & Takano. This is the first reported non-photosynthetic diatom which does not appear to be associated with the order Bacillariales. The morphology of *Tursiocola* suggests no affinity to the Bacillariales, and DNA evidence, collected by

single-cell DNA amplification techniques, confirmed that the loss of photosynthesis in *Tursiocola* was novel and unrelated to losses in the Bacillariales. The DNA sequence data also question the placement of *Tursiocola* and the epizoic diatoms *Poulinea* Majewska *et al.* and *Chelonicola* Majewska *et al.* in the Rhoicospheniaceae, suggesting that these genera are quite distant genetically from other marine “gomphonemoid” (transapically asymmetrical) raphid diatoms. We are also exploring the potential of the epizoic habitat as a model system for benthic diatom diversification. Thus far, the ease and ubiquity of obtaining cultures of the monoraphid diatom genus *Achnanthes* Bory from manatees and multiple sea turtle species in the southeastern US suggest that this genus may be an ideal model to study not only the diversification of diatoms in the epizoic habit but also across hosts.

9OR.5

EXPLOITING OMICS POTENTIAL TO FACE THE COMPLEXITY OF PLANKTON COMMUNITIES: FROM PRESENT CHALLENGES AND LIMITATIONS TO AN INTEGRATIVE PIPELINE

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Plankton drive global biogeochemical cycles and give the primary boost to aquatic food webs. The huge biological complexity characterizing this community makes the development of well-resolved, and ecologically robust models, particularly challenging. In this respect, systems ecology and evolutionary investigations focused on plankton might strongly profit from omics-based biological analyses, which would add another dimension to traditional modeling. Plankton span over several order of magnitude in size and include all kingdoms of life, including microalgae. Approaching their complexity requires a deeper and adaptive integration between data-acquisition and data-analysis, covering traditional observations (i.e., physical, chemical and biological) and information from omics (meta-barcoding, -

genomics, -transcriptomics, -metabolomics) over several time scales, validated with in-vitro and in-silico investigations and processed by advanced computational approaches, such as niche and network modeling, as well as artificial intelligence algorithms. Being aware that the integration of all the approaches above it is still in its infancy, we set up, based on this vision, a pilot-project for the NEREA Augmented Marine Observatory in the Gulf of Naples (Italy), a multidisciplinary effort started on the Darwin Day 2019 (12th February), in synergy with the Long Term Ecological Research MareChiara, and in the framework of the international program BioGeoSCAPES. Periodic, question-driven process studies within NEREA will reveal present limitations in our strategies to study plankton and will eventually lead to a new understanding of aquatic communities and to the provision of knowledge-based directions to stakeholders.

9OR.6

LEARNING TAKES TIME. SARPA SALPA’S PREFERENTIAL FEEDING ON THE INVASIVE ALGA CAULERPA CYLINDRACEA SHIFTS THROUGHOUT THE INVASION PROCESS

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The green alga *Caulerpa cylindracea* is one of the most invasive algae species in the Mediterranean Sea. However, a recent study has highlighted the role of the native fish *Sarpa salpa* as a possible control mechanism of this alga in well-preserved systems, limiting its abundance mainly at intermediate depths (between 5 and 25 m). In fact, this mechanism might be responsible for the local regressions observed in many populations some years after *Caulerpa*’s introduction. In this study, we tested whether the preference of *S. salpa* towards *C. cylindracea* changes i) throughout the invasion process, which might explain the observed delay on the grazing effect (*Caulerpa* regressions happening some years after the invasion); or ii) throughout *Caulerpa* abundances (whether *Salpa* only feeds on *Caulerpa* when this alga is a major component of the community). Feeding experiments were performed in places with high and low abundances of *Caulerpa*, both in areas that have been invaded long time ago (well-established invasion) and in places where *C. cylindracea* is

a recent component of the benthic community (initial invasion). Our results show that in places where *C. cylindracea* is ancient, *S. salpa* prefers the invasive alga to the native ones, even when *Caulerpa* abundances are low; however, in areas where the invasion is recent, *S. salpa* does not show any preference towards *C. cylindracea*, even when it is very abundant. This study shows that *S. salpa* needs some time

to get used to *C. cylindracea* before feeding on it, which might explain why *Caulerpa* population regressions always happen with some delay. Furthermore, it seems that once *S. salpa* is used to *C. cylindracea*, the preference for this alga is maintained even when its abundance is low, which means that the control mechanism is maintained through time.

10. Microbial friends and enemies of algae

10KN.1

COEXISTENCE OF GENERALIST AND SPECIALIST PARASITES IN THE PLANKTON: INTERPLAY BETWEEN DIFFERENT INFECTIVE STRATEGIES AND ENVIRONMENTAL CONSTRAINTS

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The astonishing diversity of parasites in marine plankton assemblages detected in the last decades has greatly increased interest in the ecological roles played by these organisms. Analysis of metabarcoding data obtained at a global geographic scale revealed that parasites figure among the most abundant organisms and a high incidence of parasitic links appear in interaction networks. A single plankton assemblage can contain multiple active parasites infecting the same hosts. Understanding how these parasitic species coexist is key to explaining their high diversity and quantifying the relevance of parasitic interactions to the control of hosts' populations and the flow of matter and energy through plankton food webs. Here, I present results from combined experimental and modeling approaches assessing how differences in intrinsic characteristics exhibited by sympatric generalist and specialist parasites can account for their co-existence. Infection parameters, generation times, and host ranges were compared over different temperatures to understand the effect of environmental constraints. Results indicate that specialist parasites compensate for the competitive disadvantage imposed by the dependence upon a sole host species by showing infective strategies that allow them to explore more efficiently the host population when compared to generalists (e.g., shorter generation, more resistant infective stages). Specialists also showed higher tolerance to extreme temperatures, allowing them to explore marginal conditions of their realized environmental niches, conferring an additional compensatory advantage. Interestingly, the infectivity of generalist parasites responded to temperature depended on the host. These results are particularly relevant under the current scenario of climate change, with shifts in temperature conditions potentially affecting not only the

competition between planktonic species but also their control by parasites.

10KN.2

BACTERIAL EFFECTS ON ALGAL LIFE, DEATH, AND GEOLOGY

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Micro-algae greatly influence present and past oceans. Recently we have come to realize that bacteria interact with micro-algae in various ways, ranging from pathogenicity to mutualism. My research investigates physical and chemical interactions between micro-algae and bacteria across multiple scales; from the chemical crosstalk to the influence these interactions have on the marine environment. In my talk, I will introduce *Emiliania huxleyi*, the most prevalent micro-alga in modern oceans. I will discuss the role of bacteria as hidden farmers that control the life cycle of algae; determining how fast algae will grow and how fast they will die. I will link laboratory findings to work conducted at sea and demonstrate the importance of these findings in the study of climate reconstructions.

10OR.1

MULTI-LAYERED AND CONSERVED DEFENCE MECHANISMS OF PHAEOPHYCEAE AGAINST PHYLOGENETICALLY UNRELATED PATHOGENS

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Anisolpidium ectocarpii (Oomycota) and *Maullinia ectocarpii* (Phycomyxea) are two pathogens that can parasitize several different orders of brown algae. In this study, the phenotypic changes of 43 different brown algal strains from different Phaeophyceae clades were characterized against both parasites using different microscopy techniques. We found that the host ranges of *A. ectocarpii* (Asterocladales, Desmarestiales, Ectocarpales, Laminariales, Sporochneales, Scyothamniales and Tilopteridales) and *M. ectocarpii* (Desmarestiales, Ectocarpales, Laminariales, Scyothamniales) are even broader than previously reported, and encompass one additional order (Syringodermatales) for *A. ectocarpii* and four additional orders for *M. ectocarpii* (Asterocladales, Syringodermatales, Sporochneales and Tilopteridales). Additionally, we report four defense hallmarks that are conserved in Phaeophyceae and strongly relate with resistance: i) cell wall reinforcements, namely local papilla formation beneath the infection site, sometimes accompanied of systemic cell wall thickening across the entire challenged thallus; ii) cell death particularly in challenged host cells, which after TEM evidence of swollen mitochondria, nuclear lysis and plasmodesmata obstruction suggest its relation to a hypersensitive response; iii) phlorotannin metabolism upregulation, which may suggest a defensive role as much important as against grazers and iv) Inducibility of pathogen autophagy, where pathogens turn systematically autophagic in a suicidal manner, and would correspond as a last line of defense. Additionally, most of the investigated brown algal species show v) a strong hydrogen peroxide production after being co-incubated with both pathogens, confirming an oxidative stress pathway participation in these algal-pathogen interactions. From our study, we conclude that these responses are widely conserved across Phaeophyceae, and altogether account to algal defenses. However, we observed that in several strains these responses were not strong, yet they were still resistant, suggesting that more undescribed mechanisms are contributing to the overall immunity in brown algae.

10OR.2

THE EFFECT OF LIGHT ON PHAEOBACTER GALLAECIENSIS BIOFILMS ON ULVA OHNOI (ULVALES, CHLOROPHYTA)

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Ulva spp. cultures are being used in Integrated Multitrophic Aquaculture as biofiltration systems in fish farms, both in open and recirculating systems (IMTA-RAS). The laminar thallus of *Ulva* spp. provides a niche for biofilm-forming bacteria, including those from the *Phaeobacter* genus that can reduce growth and kill fish pathogens, such as *Vibrio anguillarum*, by producing tropodithietic acid. The possibility of experimental colonization of *Ulva* spp. with *Phaeobacter* strains – previously isolated from *Ulva* species – has been proposed to control harmful bacteria in fish-algae IMTA-RAS. This would not only improve fish health but may also decrease algae fouling by detrimental bacteria, enhancing algae production. The experimental colonization of *U. ohnoi* with *P. gallaeciensis* was demonstrated and small-scale trials showed a probiotic effect of *P. gallaeciensis*-colonised *Ulva*, decreasing the mortality of *V. anguillarum*-infected turbot larvae. However, the environmental conditions for *Ulva* culture (e.g. agitation and high light intensity) could have a determinant influence on the maintenance of the *Phaeobacter* biofilms. Small-scale cultures under different light intensities demonstrated a negative influence of light on the maintenance of *P. gallaeciensis* biofilms on *U. ohnoi*. This fact was not observed when *P. gallaeciensis* biofilms were formed on a glass surface submitted to the same light regimes, indicating that light influences the algae physiology inducing chemical changes that affect *P. gallaeciensis* maintenance. Exo-Metabolomic profiling was performed by disruption-free solid phase extraction of surface metabolites and UHPLC-HR-ESI-MS analysis. Significant different features (*m/z*) were obtained from the 24 samples analyzed. Principal component analysis (PCA) showed a different behaviour of the two treatments (Presence of *Phaeobacter* or non-presence) based on the light intensity. High light intensity promoted a clear grouping of *Phaeobacter*-present samples that was not observed under low illumination. This fact indicates a potential effect of light on bacteria-mediated or bacteria-induced changes of the compound constituents on *Ulva*'s surface.

10OR.3

DETECTION AND CHARACTERIZATION OF MIXOTROPHY IN COCCOLITHOPHORES

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The current view of the marine microbial food web assumes a dichotomy between autotrophs represented by phytoplankton that drive about 45% of the global primary productivity and heterotrophic microzooplankton that consume phytoplankton. However, growing evidence indicates that an important fraction of phytoplankton are mixotrophic combining both the ability of perform photosynthesis but also to ingest by phagotrophy living prey. The functional role of marine phytoplankton is therefore more complex than previously thought. Coccolithophores (henceforth *cocco*), a group of important calcifying, unicellular algae belonging to the phylum Haptophyta, represent an important component of marine phytoplankton. Early evidence indicates that they may be able to act mixotrophically and ingest bacterial prey. In this study, we quantitatively assessed *cocco* mixotrophy. Three *cocco* species and the respectively different life cycle phases were examined, *Calyptrosphaera* sp. (haploid), *Emiliania huxleyi* (both haploid and diploid life forms) and *Calcidiscus leptoporus* (both haploid and diploid life forms), all representing important members of the *cocco* group. Cells were grown under nutrient-replete (k/5 medium) and phosphate depletes (k/5-P medium) media, in the presence of fluorescently labeled bacteria (FLB) used as prey item at the ratio of 1:20. Cells and FLB were monitored by flow cytometry and light microscopy at time points 0, 1h, 2h, 4h, 24hr and 48hr. Our results showed the active ingestion of FLB by *Calyptrosphaera* sp. and *C. leptoporus* haploid life phase, in both mediums. In contrast, *E. huxleyi* (both life forms) and the diploid phase of *C. leptoporus* did not ingest FLB. These results suggest the existence of wide variability in mixotrophic abilities across *cocco* taxa and a higher prevalence in haploid cells. We plan to expand our survey to natural populations in order to unveil the prevalence of *cocco* mixotrophy at sea.

10OR.4

SINGLE-CELL MOLECULAR ANALYSES DEMONSTRATE THE POLYPHYLY OF THE GENUS *ECTROGELLA* AND SHED LIGHT ON THE DISTRIBUTION AND ECOLOGY OF OOMYCETE PARASITES OF MARINE DIATOMS

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Photoautotrophs underpin most food webs and parasites affecting them have potential cascading effects at every level in the ecosystem. In aquatic environments, diatoms are one of the dominant groups of phototrophs and are known to be infected by oomycetes traditionally assigned to the genus *Ectrogella*. Yet the assessment of the genetic diversity, evolutionary relationships and ecological importance of this oomycete genus has been seriously hindered by the limited amount of molecular information available. Here we present results from the application of single-cell (SC) molecular analyses on marine oomycete parasites of diatoms isolated from field material. SC-PCRs, SC-whole genome sequencing and *in silico* analyses have been used to retrieve marker genes and subsequently investigate the phylogeny, distribution and parasitic dynamics of these organisms. Our findings suggest that parasitic oomycetes infecting diatoms are widespread in the global ocean and highlight local swift parasitic dynamics, likely to be one of the causes of the underappreciation of their presence by molecular ecology methods. Furthermore, metabarcoding data analysis and SC approaches alike, suggested a broad host range within diatoms, even for closely related parasite species. Finally, by strengthening the link between morphological and molecular data, SC approaches granted the possibility to reassess the classical taxonomy of *Ectrogella*. Our results highlight a high genetic diversity underlying the rather homogeneous thallus morphology observed, demonstrating the polyphyly of *Ectrogella*, and demand for the reassessment of its taxonomic treatment and for the clarification of its relationships with the genera of marine oomycetes *Anisolpidium* and *Olpidiopsis*.

10OR.5

HOW BACTERIA CAN DRIVE HAPTOPHYTES THROUGH VITAMIN B₁₂ METABOLISM

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Recent results (Nef et al, 2019) suggest that all haptophytes lineages including species of great ecological importance are auxotrophic for vitamin B₁₂. B₁₂ is produced only by some prokaryotes acting as a transmission belt between bacteria and microalgae metabolisms, and driving growth of haptophytes. Whereas synthesis of B₁₂ is well described, very few information are available on how much B₁₂ are produced by marine bacteria, in what way it becomes available to microalgae, and how a B₁₂ limitation impacts the metabolism of haptophytes. In this context, we first studied the B₁₂ production in 16 marine halomonas and phaeobacter strains. High variability was recorded between strains and comparative genomic analysis was used to highlight potential molecular mechanisms. How the bacterial B₁₂ becomes available for microalgae is now under study (excretion or lysis). We then studied the impact of a B₁₂ limitation on the metabolism of the model haptophyte *Tisochrysis lutea*. Axenic continuous cultures in chemostat were used and a full analysis of cellular carbon allocation was done. Deep comparative proteomics showed that B₁₂ limitation, compared to other nutrient limitation (nitrogen), involves a down accumulation of some enzymes of the central carbon metabolism that induces a metabolic orientation of carbon from carbohydrates towards lipids. Based on our experimental results on the minimal B₁₂ quota in *T. lutea* and B₁₂ production in the most productive bacteria, approximately one bacteria per algal cell is required to sustain microalgae growth, with the hypothesis that bacterial B₁₂ is fully available for algae. On the other hand, the carbon excreted by *T. lutea* under B₁₂ limitation would be enough to sustain such bacterial load. These results give new insight into how the interaction between haptophytes and B₁₂ producing bacteria can be shaped

and on the influences of these interactions on the metabolism of haptophytes.

10OR.6

WHY THE RED ALGAL HOST *PYROPIA YEZOENSIS* RESPOND DIFFERENTIALLY TO THREE *OLPIDIOPSIS* SPECIES?

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Olpidiopsis blight caused by Oomycete pathogens is one of the most serious threats for *Pyropia* farming in East Asia. Four closely related *Olpidiopsis* species which infect *Pyropia* blade have been identified so far; *O. porphyrae* in Japan, *O. pyropiae* and *O. porphyrae* var. *koreanae* in Korea, and *O. porphyrae* var. *scotiae* in Scotland. We compared infection pattern of three *Olpidiopsis* species which showed different infectivity towards Korea *Pyropia yezoensis*. *O. pyropiae* showed the strongest infectivity from the initial stage of infection and spread to the whole blade much faster than the other two. Apoptosis of host cell was inversely related to the infectivity of infection. The apoptosis was most prominent in the host infected with var. *scotiae*, which showed the weakest infectivity. The color of infected host cell changed differently when it was infected by *O. pyropiae* (yellow), *O. porphyrae* (green) and var. *scotiae* (green) suggesting that the chloroplasts respond differently to each pathogen. The infection of *Olpidiopsis* pathogen was mediated by the lipid-raft of the host cell membrane. When the PI3K inhibitor, LY294002, and Wortmannin, was treated prior to infection the infection rate decreased in all pathogens. These results suggest that three species of *Olpidiopsis* infect the host cell using similar lipid-raft mediated process, but the apoptosis of the host cell after the initial infection affects the spread of disease in the infected blade.

11. Biotechnological applications of algae: algae as a source of food, drugs, energy

11KN.1

RECENT ADVANCES IN MICROALGAL BIOTECHNOLOGY WITH AN EMPHASIS ON BIOFILM CULTIVATION

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Microalgae are at the center of a very dynamic activity both in terms of research and industry. Current production systems are mainly based on suspension culture units using large volumes of water such as in raceway ponds or photobioreactors. The relatively low biomass concentration observed within these planktonic-based cultures leads to high water requirements and expensive harvesting costs, alone covering for 30% of the energy cost of production. The recent technological breakthrough of biofilm-based algal cultivation systems offers an alternative that could solve some of these problems. Indeed, extracellular polymeric substances (EPS) secreted by microalgae bind cells and allow them to adhere to solid materials. Biofilms usually consist of autotrophic microalgae associated with other heterotrophic microorganisms such as bacteria that play a crucial role in their initiation and development. Well-developed microalgal biofilms may vary from a few to several hundred micrometers and can be harvested by simple scraping, thus providing a considerable energy gain of approximately 50% in comparison with suspension-based cultivation. The other advantage, which lies in better access to light, allows productivities 300% higher than conventional production systems. In this context, this presentation will describe recent advances on microalgal biofilm cultivation systems, their main uses to date, and their process optimization and modeling for better growth performance and controlled biochemical composition. As part of the Phyto'Recolt project (GRAINE-ADEME, France,

2018-2020), we have developed various biofilm cultivation strategies in response to light, salinity, carbon sources, shape configuration, rotating speed and harvesting frequency using rotating algal biofilm (RAB) systems. The RAB systems in use have been run during a full year keeping contamination to a very low level, achieving ground productivity of 18 g/m²/day when the biomass was collected every 10-12 days.

11KN.2

TRANSITION FROM AQUATIC TO TERRESTRIAL SYSTEMS: A PARADIGM CHANGE IN ALGAE BIOTECHNOLOGY?

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In the field of algae biotechnology, pressing challenges are the optimization of algae production, the corresponding process engineering and the scale-up of photobioreactors. The main focal points are the reduction of fermentation limitations in terms of mass transfer (in particular CO₂ & O₂ gas), thermal stability, photoinhibition and the efficiency of energy and resource investments. So far, almost all cultivation technologies are realized under submerged conditions with aquatic microalgae. The biotechnological implementation of terrestrial microalgae, however, shows several physiological and procedural advantages for efficient production in biofilm-photobioreactors (Lakatos & Strieth 2017, Strieth et al., 2017). Their outstanding performance and significant benefits for biotechnology may remove some of the current limitations and provide new concepts in biotechnological fermentation. How terrestrial

microalgae can contribute to the technological and economic improvement of microalgae biotechnology is discussed in terms of biodiversity and physiological range of new concepts and applications. The focus is on important key processes of emerge photo-fermentation with terrestrial microalgae: thermostability, light adaptation and desiccation tolerance.

11OR.1

CHLAMYDOMONAS CAN BE CONVERTED INTO AN EFFICIENT AND CONTINUOUSLY WORKING GLYCOLATE-PRODUCTION MACHINE WITHOUT FORMING BIOMASS

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Glycolate can be synthesized in autotrophic cells under high temperatures and Ci-limitation via oxygenation of ribulose-1,5-bisphosphate by photorespiration. In unicellular algae, photorespiration consumes reductants, ATP and emits CO₂ and is, therefore, an inhibitory process for biomass production. However, if the cells are manipulated in a way that they perform carboxylation/oxygenation in a ratio of 2, they become glycolate producing “cell factories”. However, the efficiency of this pathway is high only when the C2 cycle is blocked, glycolate excretion becomes the only pathway of photosynthetic carbon flow. Here we show that it is possible to run a culture of *Chlamydomonas* under specific cultivation conditions to establish a constant and long-term stable glycolate excretion during the light phase. The results demonstrate a constant high C-assimilation and an extremely high rate (82%) of assimilated carbon transferred into glycolate biosynthesis without losses of function in cell vitality for weeks. Moreover, the glycolate accumulation in the medium is high enough to be directly used for microbial fermentation without any changes in the composition of the medium allowing complete recycling after glycolate consumption by the fermentation process.

11OR.2

PROTEIN SCAFFOLDING FOR NEXT-GENERATION CYANOBACTERIAL CELL FACTORIES

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Cyanobacteria are versatile photosynthetic microorganisms with great potential as sustainable cell factories. However, there are several major limitations that will have to be overcome to make cyanobacteria a competitive alternative to existing biotechnological chassis. The Cynthetica project, funded by the Horizon 2020 research and innovation programme of the European Union, uses a synthetic biology approach to tackle one of the principal limitations in cyanobacteria: low product yields. Our goal is to engineer designated locations and compartments for product synthesis within the cell. To achieve this, we are engineering cytoplasmic protein-based structures serving as a scaffold for enzymes of a biosynthetic pathway. We have expressed a structural scaffold protein in two different cyanobacterial species, the widely used model organism *Synechocystis* sp. PCC 6803 and a recently described, fast-growing cyanobacterium *Synechococcus elongatus* UTEX 2973. Our preliminary results are promising to show intracellular, *de novo* structures formed *in vivo*. Here, we will discuss the implications of this technology for the next generation of cyanobacterial cell factories.

11OR.3

MARINE MACROALGAE AS FUNCTIONAL FOOD TOWARDS GENOME PROTECTION – FROM AQUACULTURE TO HUMAN HEALTH

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Marine macroalgae are considered a source of bioactive compounds, highlighting them as a functional food. Their genoprotection ability is raising interest, but they are underexplored regarding nutraceutical and

biotechnological applications, namely as food or feed (e.g. aquafeeds) items. Hence, our goals concerned the evaluation of the antigenotoxic potential of *Ulva rigida*, *Fucus vesiculosus* and *Gracilaria gracilis* (i) on fish (*Sparus aurata*), through a diet supplementation, and (ii) on mice (*Mus musculus*, FVBn strain), both directly and indirectly (using fish as a vehicle of algae phyto-components), complemented by basic haematological parameters. For that purpose, two trials were designed. The first concerned fish diet supplementation with a mix of the three algae (5%) for 2 months, after which fish were injected with the genotoxicant cyclophosphamide (CP – 20 mg kg⁻¹ b.w.). Four days later, blood was collected to perform erythrocytic nuclear abnormalities (ENA) and erythrocytic maturation index (EMI) assays. The second trial concerned mice diet supplementation with the algae mix (5%) (A), muscle of *S. aurata* (10%) fed with an aquafeed supplemented with the same algae mix (FA), or muscle of *S. aurata* (10%) fed with standard aquafeed (F). After 1 month, mice were injected with the genotoxicant methyl methanesulfonate (MMS – 40 mg kg⁻¹ b.w.). Two days later, blood was collected, and comet and micronuclei assays were performed, alongside microhematocrit. Algae supplementation barred the CP-induced chromosomal damage and enhanced the erythropoiesis rate in fish. Regarding mice, the different diets (F, FA and A) prevented MMS-induced genetic damage (comet assay) while the A diet was the most effective considering chromosomal damage (micronuclei assay) (A<FA<F). Haematocrit values remained unaltered. Overall, the genoprotection capacity of *U. rigida*, *F. vesiculosus* and *G. gracilis* was demonstrated, suggesting their inclusion in aquafeeds (to promote genome integrity of farmed fish and as an indirect route of algae phyto-compounds) and in human nutrition.

11OR.4

THE EFFECT OF LED LIGHTING ON NIGHT BIOMASS LOSSES OF NANNOCHLOROPSIS OCEANICA GROWN IN OUTDOOR PILOT SCALE RACEWAYS

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Since night biomass losses can have a significant impact upon the productivity of microalgal cultures, the introduction of artificial lighting during the night cycle is considered as a promising solution to enhance biomass productivity. Moreover, artificial light is able to supply a higher photon flux in shaded areas of culture systems, which is a key limiting factor for algal growth. Our goal was to prevent a bioenergetic shift of the cells from photosynthesis to a respiration-only stage by using LEDs to artificially extend the light period. To this end, we used *Nannochloropsis oceanica* cultures grown in 300-L outdoor pilot-scale raceway ponds operated in batch under a controlled circadian cycle using three conditions: 1) LEDs turned on for 24 hours; 2) LEDs turned on during the night; 3) Without LEDs (control). Sampling was performed daily, before the light period and again at the end of the day to monitor the growth performance and nightly biomass loss due to respiration. Cell fitness (chlorophyll fluorescence) and biosynthesis of pigments (chlorophyll and carotenoids), lipids, proteins, carbohydrates, and fatty acids were also monitored. Higher growth performance was observed when cells were LED-lit for 24 hours, followed by cultures LED-lit at night, as compared to control cells (no LEDs). Conversely, control cells showed the lowest values of non-photochemical quenching. Daily variations in biochemical composition were also observed, namely in the total lipid content and in the fatty acid profile when morning and evening samples were compared. The effect of LEDs on cell respiration in the dark and what strategies microalgal cells use to deal with the extra photons received as well as the daily fluctuation of target metabolites in *N. oceanica* cultures will be discussed.

11OR.5

FRESHWATER MACROALGAE AS A SUSTAINABLE RAW MATERIAL FOR THE PRODUCTION OF AGROCHEMICALS

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Freshwater macroalgae, being the result of eutrophication of water reservoirs, constitute an unused source of the biomass in many regions around the world. Due to their unique chemical composition (micro- and macroelements, amino acids, vitamins, polysaccharides, antioxidants etc.) and properties they can be used as a raw material for the production of agrochemicals. In the research, different

methods were applied in order to obtain valuable products from the freshwater alga – *Cladophora glomerata*. Biosorption process was used to produce biological feed additives or components of fertilizers with microelements. Enriched algae can have beneficial effects on the growth and health parameters of animals and plants. Ultrasound-assisted extraction enabled the production of algal extracts that can be used as biostimulants of plant growth that are known to enhance the nutrition efficiency, tolerance to the biotic and abiotic stress and the crop yield and quality. Additionally, in the research, the effect of algal extracts applied individually or in the combination with different physical factors (e.g., static and alternating magnetic field; near-infrared radiation) on the germination of seeds (soybean, carrot) was examined. Pyrolysis of freshwater macroalgae resulted in the production of biochar which when applied to the soil can influence its properties, increase plant productivity and has a positive impact on the environment. These studies indicate the importance of algae for agriculture and the multitude of applications for this biomass.

11OR.6

HASLEA OSTREARIA-LIKE DIATOMS OR ‘LA VIE EN BLEU’

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The genus *Haslea* is a taxonomic unit of marine pennate diatoms defined by R. Simonsen in 1974, which refers to fusiform or lanceolate cells, the frustule of which is made of two valves, each presenting two layers assembled in a typical bi-layered structure. *Haslea* frustule valves present characteristically a totally different aspect when comparing their external and internal surfaces. The former presents continuous longitudinal fissures, the latter is perforated by areolae, square to rectangular openings, forming grate-like bars. The genus *Haslea* type species is *Haslea ostrearia*, a tychopelagic/benthic/epiphyte organism that produces marennine, a water-soluble blue pigment. Marennine is a bioactive compound with antioxidant, antimicrobial, and allelopathic effect against other

diatoms. It is also responsible for the greening of oysters in refining ponds in Western France, however, the greening of bivalves or other invertebrates' gills occur naturally elsewhere in the world (e.g., USA, Australia). For decades, any record worldwide of a blue diatom or green-gill bivalves was assigned to *H. ostrearia*. However, recent works on the blue *Haslea* using scanning electron microscopy and molecular approaches have enlightened unexpected biodiversity of this taxon, with the description of new species, *H. karadagensis* collected in the Black Sea, *H. provincialis* in the Mediterranean Sea, and *H. nusantara* in the Java Sea. Some progress has been made regarding our knowledge about the formation of blue *Haslea* blooms in natural environments, the possible consequences of the amount of marennine-like pigments produced and released in seawater, and the chemical nature of these pigments. NMR spectroscopy has revealed that the scaffold of marennine is a complex polysaccharide, probably with an aromatic aglycon as chromophore. The composition of the carbohydrate has been determined quantitatively after hydrolysis.

11OR.7

SCREENING OF ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITY OF CYSTOSEIRA AMENTACEA VAR. STRICTA EXTRACTS

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Marine macroalgae represent a valuable resource in pharmaceuticals, cosmetics and food industry. Brown macroalgae are rich in phenolic compounds that exert several promising bioactivities, particularly, phlorotannin compounds are involved in antioxidant and anti-inflammatory processes. In the present study, the in vitro antioxidant and anti-inflammatory activities of *Cystoseira amentacea* var. *stricta* were evaluated using water/ethanol (CaEt) and dimethyl sulfoxide (CaD) extraction methods. The antioxidant capacity was measured by the 2,2-diphenyl-1-picrylhydrazyl (DPPH), hydroxyl (OH·) and nitrite (NO·) radical scavenging activities, as well as the Ferric reducing antioxidant power (FRAP) by spectrophotometric tests. The antioxidant and anti-inflammatory potential was also evaluated using two different cell lines: RAW 264.7 murine leukemia cells and L929 murine fibroblasts. Additionally, the total

phenolic and flavonoid content of both extracts was quantified. Both extracts revealed significant antioxidant activity in all spectrophotometric tests, especially, for DPPH test and NO· radical with a 100% and > 80 % of scavenging activity, respectively. In the two cell lines tested, the two extracts were able to quench completely intracellular ROS production stimulated by incubation with 200 µM H₂O₂ for 2 h. Regarding the anti-inflammatory activity, both extracts were able to inhibit the production of typical pro-inflammatory mediators in RAW 264.7 macrophage cell line stimulated with 100 ng/ml LPS for 24h. qPCR analysis revealed inhibition of 83% of Cyclooxygenase-2 gene over-expression and a 73% inhibition of Interleukin-1β over-expression caused by CaD extract. In the same cells, stimulated by LPS, over-expression of the NO synthase enzyme was significantly inhibited by > 90% using both extracts. Finally, the total phenolic and flavonoid content was higher for CaD extract (75.42 and 603.93 g/mg extract), which, overall, reported a slightly higher antioxidant and anti-inflammatory capacities. In conclusion, these data demonstrate a valuable and exploitable pharmacological potential of *Cystoseira amentacea* var. *stricta* extracts to be further pursued.

11OR.8

EFFICIENT SECRETION OF A LYTIC POLYSACCHARIDE MONOOXYGENASE IN A FAST-GROWING CYANOBACTERIUM

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Cyanobacteria have great biotechnological potential due to their ability to use water as an electron donor, CO₂ as a carbon source and light as a primary energy source to express heterologous enzymes. In particular, *Synechococcus elongatus* UTEX 2973 is a novel fast-growing cyanobacterium that has gained attention as a new biotechnological chassis. To establish this strain as a host for heterologous protein production we demonstrate the expression and secretion of industrially relevant *TfAA10A*, a lytic polysaccharide monooxygenase (LPMO) from the gram-positive bacterium *Thermobifida fusca*. We generated two cyanobacterial transformants expressing the *TfAA10A* – one with the native, Sec-targeted, signal peptide (*TfAA10A*) and a second with a heterologous, Tat-targeted, signal peptide (TorA-*TfAA10A*) replacing the native signal peptide. Results showed that the

majority of the TorA-*TfAA10A* was found unprocessed in the plasma membrane with only a small fraction of the mature protein ultimately translocated to the periplasm. The native, Sec-targeted, *TfAA10A* was recognized by the cyanobacteria secretion machinery and virtually all the protein was directed to the culture medium with a secretion yield estimated at 779 ± 40 µg L⁻¹. Additionally, *TfAA10A* was shown to be correctly folded and active on the model LPMO substrate phosphoric acid swollen cellulose. This proof-of-concept study constitutes the first demonstration of the full secretion of an industrially relevant enzyme in *S. elongatus* UTEX 2973¹. Cyanobacterial secretion may have applications in whole cell biocatalysis, bioremediation and carbon capture.

11OR.9

INCREASING BIOMASS AND LIPID PRODUCTIVITY BY ENGINEERED *PHAEODACTYLUM TRICORNUTUM*

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Diatom *Phaeodactylum tricornutum* is one of the best organism to study bio-resources. Its biomass and lipid are representative bio-resources. Generally, the culture conditions for the accumulation of lipids in the cells inhibits cell growth and biomass, but normal growth conditions for algae do not trigger lipid accumulation. So we attempted to find the strategy that could produce both high biomass and lipid simultaneously. Pyruvate is a precursor of various amino acids, fatty acids, and so on. Cells have certain transporters which transport pyruvate sodium-dependently or proton-dependently. Pyruvate transporter, especially plastid-localized type, is known to be involved in lipid synthesis, transporting pyruvate into plastid and providing a precursor for fatty acid synthesis. BILE ACID:SODIUM SYMPORTER FAMILY PROTEIN 2 (BASS2) is a plastid-localized pyruvate transporter recently has been identified in plants. So we searched BASS2 homolog from *P. tricornutum*. Then, we cloned full-length cDNA sequences of pyruvate transporter (PtPTP) from *P. tricornutum* by 5'/3'- RACE (rapid amplification of cDNA ends), and sub-cloned into an expression vector, pPhat-EF2, which contains an endogenous constitutive promoter. To determine the localization of PtPTP, transformants expressing pyruvate transporter tagged with EYFP were generated.

Fluorescence microscopy showed pyruvate transporter localized to the plastid. Next, we obtained two transformants highly overexpressing pyruvate transporter. To examine the effects of overexpressed PtPTP, we analyzed their growth rate, biomass and lipid contents. As a result, we confirmed that the transformants not only have shown better growth rates than that of wild type, but also biomass increased as much as 13% to 22%, lipid contents increased as much as 11% to 30%. These results suggest that the overexpressing plastid-localized pyruvate transporter increases biomass production and lipid contents.

11OR.10

RAPID SCREENING TEST TO ESTIMATE MICROALGAE VIABILITY USING PHOTOSYNTHESIS MEASUREMENTS

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Microalgae viability is influenced by several environmental variables, such as temperature, irradiance, nutrient availability, salt concentration, etc. Various microalgae tolerate a different range of these variables; thus fast pre-screening method is of interest to control pilot or large scale cultivation. Some microalgae grow between 15 and 40 °C (e.g. some *Chlorella* strains) while the other, namely marine strains require more accurate temperature regulation. We aimed to work out a rapid procedure for fast pre-screening of growth regime based on photosynthesis measurements. Three techniques were employed to monitor photosynthetic activity: measurement of photosynthetic oxygen evolution as well as two chlorophyll fluorescence techniques – saturating pulse analysis of fluorescence quenching and fast fluorescence induction kinetics. To verify this procedure several microalgae strains were used, e.g. *Chlorella*, *Scenedesmus*, *Synechocystis*, *Nostoc* etc. Such pre-screening tests taking about one day make it possible to estimate suitable growth regime (temperature, light demand) of microalgae strains. Then, more precious growth trials can be carried out to verify strain suitability for biomass production. On the other hand, activity-controlled nutrient deprivation may induce accumulation of valuable compounds, although over-stressed culture can be lost rapidly. For example,

increased salt concentration up to some limit may suppress contamination without substantial growth reduction.

11OR.11

EFFECT OF CULTURE MEDIUM ON THE BIOMASS PRODUCTION AND BIOCHEMICAL COMPOSITION OF SELECTED MALAYSIAN THRAUSTOCHYTRIDS

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Thraustochytrids are a diverse group of non-photosynthetic marine protists that are commonly found in marine and estuarine habitats including mangroves. They produce large amounts of lipids, have a fast growth rate and high biomass production. More recent studies have shown that in addition to being an important feedstock for biofuel production, thraustochytrids also produce other high-value bio-products such as omega-3 long-chain polyunsaturated fatty acids (PUFAs) particularly docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA); carotenoids like astaxanthin, squalene, and sterol, which further contribute to the commercialization value of thraustochytrids. This study aimed to determine the biomass and lipid productivity of *Aurantiochytrium* UMACC-T023 under different culture media (carbon and nitrogen sources and concentrations). *Aurantiochytrium* UMACC-T023 was isolated from the Port Dickson mangrove, west coast Peninsular Malaysia. The biomass productivity of UMACC-T023 increased from 0.54 ± 0.09 to 1.39 ± 0.26 g/L/D and lipid productivity increased from 19.93 ± 9.34 to 31.27 ± 2.45 mg/L/D, when the glycerol concentration was increased from 1% v/v to 8% v/v in the medium. The biomass productivity of UMACC-T023 increased from 0.13 ± 0.05 to 0.43 ± 0.09 g/L/D and lipid productivity increased significantly (P < 0.05) from 10.91 ± 4.93 to 45.47 ± 8.17 mg/L/D in the polypeptone concentration was increased from 0.1% w/v to 2% v/v in the medium. DHA was produce in highest abundance. Highest total carotenoid content of UMACCT-023 (1.1837 µg/ml) was reported in culture medium contain 2% w/v yeast extract, 1% w/v glucose, 0.1% polypeptone, pH7.0

and 30ppt. *Aurantiochytrium* UMACC-T023 had potential as a source of biochemical for the nutraceutical and cosmeceutical industry.

11OR.12

HETEROTROPHY AS A TOOL TO ENABLE LARGE SCALE MICROALGAE CULTIVATION FOR CO₂ MITIGATION

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Mass culture of microalgae is a promising strategy for CO₂ sequestration. However, its autotrophic scale-up process is slow, requires a large occupancy area, and depends on weather conditions, being also prone to culture collapse and to the appearance of unwanted

contaminants. Thus, it is challenging to reproduce the CO₂ mitigation values obtained at smaller scales and prove its feasibility at an industrial scale. To solve this problem, a two-stage scale-up process was developed: heterotrophically *Chlorella vulgaris* cells grown in fermenters (1st stage) were used to directly inoculate an outdoor industrial autotrophic microalgal production unit with multiple flat panel photobioreactors (2nd stage). Cells were grown heterotrophically adapted readily to autotrophic growth conditions without any measurable difference as compared to autotrophic inocula. Biomass concentrations of 174.5 g L⁻¹ were achieved in a 5-L fermenter during the scale-up process using the novel heterotrophic route. Inocula grew in 0.2- and 5-m³ fermenters were later used to seed several outdoor 100-m³ tubular flow-through photobioreactors. The biomass originated from the heterotrophic pathway reached 23.98 ± 1.57 mg g⁻¹ DW of chlorophyll and 52.18 ± 1.30 % of DW of protein within 5 days of autotrophic growth. These values are in accordance with the reported range for exclusive autotrophic *Chlorella vulgaris*. In addition to providing reproducible, high-quality inocula, this two-stage approach led to a 4-fold and 12-fold decrease in scale-up time and occupancy area of the facilities used for microalgal culture scale-up.

12. Planktonic and benthic Harmful Algal Blooms: a threat for human health and resources

12KN.1

ROLE OF ENVIRONMENTAL FACTORS ON DYNAMICS AND TOXIN PRODUCTION OF BENTHIC HARMFUL ALGAL BLOOMS

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Coastal waters are widely exploited by human activities and, in tropical regions, are traditionally threatened by outbreaks of toxic benthic dinoflagellates (*Gambierdiscus*, *Ostreopsis*, *Coolia* and *Prorocentrum*). Some species are responsible for severe illnesses, caused by consumption of contaminated seafood or the formation of toxic bio-aerosols. In the last decades, benthic Harmful Algal Blooms (bHABs) have increased in frequency, intensity and geographic distribution in temperate areas as well, probably due to a combination of natural and human-driven effects, including climate change. Environmental factors, both abiotic and biotic, are known to influence several aspects of bHABs, from their formation, toxin production and accumulation to their effects on the environment and human health. While in tropical regions benthic dinoflagellates are common members of microphytobenthos communities throughout the year, in temperate areas their maximum abundances are associated with the high water temperatures typical of the summer-autumn period. However, many studies have recently highlighted that other environmental factors besides temperature affect growth and toxin production of benthic dinoflagellates, such as hydrodynamics, depth, nutrient concentrations, substrate availability and allelochemical interactions, with different effects depending on the phases of the bloom. The availability of both inorganic and organic phosphorus seems to play a key role in triggering and maintaining blooms of benthic dinoflagellates, particularly in P-limited areas. Interactions with benthic diatoms and macroalgae, detected both in laboratory and field studies, suggest that the production of some allelochemicals, such as Polyunsaturated Aldehydes (PUAs), could affect bloom dynamics. Toxin production is modulated by cellular physiological status, which is affected by a wide range of factors, including

several environmental stressors (which, however, are not necessarily the same that stimulate cell proliferation). This fact could represent an additional difficulty in the management of bHAB phenomena.

12KN.2

CYANOBACTERIAL BLOOMS IN LAKES: ECOLOGY, PREVENTION AND CONTROL

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In this overview presentation, the ecology and eco-physiological traits of different cyanobacterial groups will be discussed. Many cyanobacteria produce toxins which can harm aquatic animals, but also dogs, cattle and humans. Cyanobacterial blooms often cause problems in lakes and reservoirs for the use of the water for drinking water and recreation purposes. Prevention of blooms requires that external and internal sources of nutrients are managed to levels where development of cyanobacterial blooms is restricted. A system analysis of the lake, in particular its morphometry, water and nutrient balance are needed. Preventing toxic cyanobacterial blooms by reducing internal and external nutrient loads is a preferable method in lakes and reservoirs that cope with these blooms, yet it is usually a long-term process. Control of blooms, in which their presence is reduced to a level where they no longer pose a risk through additional measures such as biomanipulation or artificial mixing, demands an understanding of the key ecological traits of the dominant cyanobacteria taxa, and adequate design and execution of the management methods of choice. Cyanocides like hydrogen peroxide can be used to reduce the cyanobacterial biomass since cyanobacteria typically show a higher

sensitivity to hydrogen peroxide than other groups of aquatic biota and this control agent does not leave any harmful residuals due to its breakdown into oxygen and water. Treatment with H₂O₂ can be a rapid, highly effective and low-cost method to combat cyanobacterial blooms, but the duration of the post-treatment period with no or low numbers of cyanobacteria varies among lakes. The choice of a method for prevention or control of cyanobacterial blooms for a specific lake is dependent on its lake morphology, water and nutrient balance, dominant cyanobacterial species, and economical aspects.

12OR.1

EFFECT OF SALINITY AND TEMPERATURE STRESSES ON DMSP AND PSTS CONTENT IN *A. MINUTUM* AND *A. CATENELLA*

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Microalgae of the genus *Alexandrium* are able to form blooms and may have health and climate impacts because they produce two molecules of key-stone significance: saxitoxin and analogues known as paralytic shellfish toxins (PSTs) and the dimethylsulfoniopropionate (DMSP), an organosulfur compound. The production of DMSP and PSTs may be influenced by various environmental conditions such as salinity, temperature, light, turbulence and nutrient concentrations. Interestingly, under certain turbulence conditions, it was observed that the biosynthetic pathway of DMSP was favored and that PSTs metabolic pathways were not, suggesting a possible link between the metabolic pathways of DMSP and PSTs. In a context of climate change that predicts an increase in the frequency and intensity of *Alexandrium* blooms, it appears important to better understand the impact of temperature and salinity variations on the production of DMSP and PSTs, and potential interactions between both metabolites. The effect of changing salinity and temperature conditions on DMSP and PSTs were investigated in batch cultures of two French strains of *A. minutum* (AM1231 and AM1232) and two strains of *A. catenella* (IFR-ACA-15 and H8-4). The strains were subjected to three salinity shocks (-15 PSU, +8

PSU, +13 PSU) under two conditions of temperature (25°C and 18°C the control condition). During the 48-hour experiment, monitoring of photosynthetic activity, cell density, biovolume, toxins and DMSP were recorded. The conditions leading to significant variability in DMSP and PSTs will be tested by RNA-seq analysis to identify genes likely involved in DMSP and PSTs biosynthesis in *A. minutum* and *A. catenella*. This study provides an important first step towards the understanding of the influence of two abiotic factors on the DMSP and toxin production and gives new insights on the potential contribution of these molecules in a changing ocean.

12OR.2

SEASONAL MONITORING OF CYANOBACTERIA AND CYANOTOXINS BY FLOW CYTOMETRY AND LIQUID CHROMATOGRAPHY COUPLED TO TANDEM MASS SPECTROMETRY, IN TWO LOWLAND FRESHWATER RESERVOIRS SITUATED IN SOUTHERN BRITAIN

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Cyanobacteria blooms can adversely affect freshwater resources by cell super abundance and the release of potent toxins. *Microcystis* is a genus of unicellular and colony-forming coccoid cyanobacteria, which are ubiquitous in freshwaters across the globe. Microcystins are a group of toxic cyclic heptapeptides produced by *Microcystis* and other genera, which can impede mammalian liver function and have been associated with tumor promotion. An increase in the occurrence and severity of blooms has been linked to anthropogenic climate change and eutrophication. The prediction of cyanobacterial blooms is an important objective of monitoring regimes and paramount in freshwater resource management. Longham Lakes are two connecting reservoirs, varying in age and management strategies, situated on the south coast of Britain. Water samples were taken approximately every 10 days over a 13-month study period (05/2016 to 05/2017), simultaneously biological and chemical water measurements were recorded by the multi-parameter probe. Cyanobacterial cells

were differentiated and enumerated by established light microscopy and newly developed flow cytometry methods. Microcystin variants were qualified and quantified by a novel UHPLC-MS/MS, which was suited to high throughput regulatory testing. Cyanobacterial cell counts exceeding UK threshold levels of >20,000 cells mL⁻¹ were recorded on four occasions. Toxins were detected in both reservoirs, but concentrations were significantly higher in one and did not correspond with the highest levels of *Microcystis* cells. Microcystin levels did not exceed the WHO medium health threshold of 20 µg L⁻¹ although low threshold values (1.0 µg L⁻¹) were detected. Monitoring data indicated complex bottom-up and top-down control mechanisms in the moderation of cyanobacterial taxa abundance and population structure, the latter potentially mediated by the presence of omnivorous fishes. Application of basic general linear modeling to the dataset indicated that approximately 60-65% of the variability could be explained by combined independent abiotic and biotic variables indicating the future applicability of this approach.

12OR.3

CULTURE OF PREY ORGANISMS OF *DINOPHYSIS* HELPS EXPLAIN WHY ITS OCCURRENCE ALONG THE FRENCH ATLANTIC COAST DOES NOT SHOW TRENDS OVER A 20-YEAR PERIOD

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Several species of the genus *Dinophysis* are known to produce lipophilic toxins and form harmful algal blooms (HABs) globally. Such toxins can be accumulated in suspension feeding bivalves and may intoxicate consumers of contaminated shellfish. This study is part of the European project CoCliME which, among others, aims

at assessing the potential impact of global change key factors on growth, distribution and toxicity of toxic species of the genus *Dinophysis*. It is also important to understand the ecology of prey organisms of this obligate mixotroph since *Teleaulax amphioxeia* and *Mesodinium rubrum* have been shown to be sources of plastids for *Dinophysis*. Optimal growth and physiology of *T. amphioxeia* as a prey model were determined without acclimation using a factorial design for temperature, irradiance and pH in an experimental set-up with 15 combinations. The model predicted a higher growth rate of *T. amphioxeia* at high light intensity (400 µmol photons m⁻² s⁻¹), and in more acidic pH (7.6) and warmer temperature (17.6 °C) than current conditions in French Atlantic waters. Subsequently, *M. rubrum* was fed with *T. amphioxeia* acclimated at different conditions to investigate potential impacts of prey quality and quantity on the physiology of the predator. *M. rubrum* growth appeared to be more constrained by the quantity of prey provided than by the pigment content of the prey. Monitoring over a 20 year monitoring period of *Dinophysis* and its toxins along French Atlantic coasts did not show a clear time trend. While no significant increase in the occurrence of *Dinophysis* was observed over this period, the experimental studies indicate a potential for a significant increase of *T. amphioxeia* and *M. rubrum* occurrences in future climate conditions, which in turn may lead to increased occurrence of *Dinophysis* spp., already significantly impacting the European shellfish industry.

12OR.4

STUDY OF GROWTH AND TOXIN PRODUCTION OF CULTURED *GAMBIERDISCUS* SPP. STRAINS FROM MACARONESIA

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Ciguatera poisoning is a food-borne illness mostly caused by consumption of fish contaminated with polyether toxins known as ciguatoxins (CTXs).

Originally known as a tropical disease, Ciguatera is being increasingly reported from areas previously not considered endemic, namely in the North-Eastern Atlantic. The epi-benthic dinoflagellates *Gambierdiscus* and *Fukuyoa* are considered to be the causative agents of Ciguatera. Furthermore, these genera have recently been shown to have an increasing number of species, also discovered in areas where it had not been observed before, namely the Canary Islands. The study presented here is part of the MIMAR project (MAC/4.6D/066) aiming at the characterization of hitherto unidentified CTXs from the Macaronesian region. A total of 54 strains of the genus *Gambierdiscus* were recently isolated from field samples collected from Macaronesia (species identification is ongoing). Five strains from the Canary Islands (G45, G85, G92, G100 and G141) were used in this study for evaluation of cell growth and toxin production. Cell growth was performed in 75 cm² culture flasks under the same culture conditions, using both semi-continuous and batch cultures. Two brands of the culture flasks (VWR® and Corning®) and four different treatments of the bottom surface (non-treated, treated, cell bind-treated and ultra-low-attachment treated) were evaluated for their influence on the growth of the strains. All the conditions tested were suitable for culturing *Gambierdiscus* spp., although some slight differences were observed in growth rates and cell behavior. Triplicate samples obtained by semi-continuous cultures of the five strains (exponential growth phase) were used for screening their CTX-activity using the neuroblastoma neuro-2a assay. Preliminary results suggest that G45 and G92 are the most CTX-producing strains. Further studies will focus on culture scale-up and bioguided fractionation of the most toxic strains in combination with high-resolution mass spectrometry to pinpoint known and/or previously undescribed toxins.

12OR.5

EFFECT OF NUTRIENTS ON NATIVE AND ALIEN NUISANCE CYANOBACTERIA STRAINS FROM TEMPERATE LAKES AND THEIR INTERSPECIES COMPETITION

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The development of cyanobacteria communities in freshwaters is substantially related to the number of simultaneously acting environmental conditions. Nutrient enrichment plays an important role in causing toxic blooms of cyanobacteria and also may modify dominant species composition. Experimental approaches are a very helpful tool to understand the response and role of a particular species in an ecosystem. Therefore, eight strains of native (*Planktothrix agardhii*, *Aphanizomenon gracile*) and alien (*Chrysoosporum bergii*, *Sphaerospermopsis aphanizomenoides*) cyanobacteria were selected to test their growth rate, production of cyanotoxins and oligopeptides, and to assess interspecies competition in response to the increased availability of nutrients and temperature changes. The general linear model showed that concentrations of inorganic phosphorus (IP), inorganic nitrogen (IN) and their ratio significantly positively affected the growth of all tested cyanobacteria. The growth rate depended on the particular strain, species and species origin also. The highest growth rates were found for *A. gracile* ($0.43 \pm 0.08 \text{ day}^{-1}$) and *S. aphanizomenoides* ($0.40 \pm 0.12 \text{ day}^{-1}$), the lowest – for *P. agardhii* ($0.14 \pm 0.25 \text{ day}^{-1}$) at all tested nutrient concentrations. Low IP concentrations and IN:IP ratio inhibited the growth of non-diazotrophic *P. agardhii* cyanobacteria. The highest amount of prevailing toxins dmMC-RRm, dmMC-LR variants and other oligopeptides (anabaenopeptins, aeruginosins, cyanopeptolins) in *P. agardhii* were detected at the lowest IP concentration. A clear relationship between STX production by *A. gracile* and nutrients was not revealed. Nutrients concentration had a greater effect on species competition than temperature and species origin. Native *P. agardhii* and *A. gracile* competed mainly for nutrients whereas co-cultured native and alien species acted differently. *P. agardhii* growth was suppressed by alien *S. aphanizomenoides*. Nutrients and temperature rather than interspecies competition were important for *P. agardhii* and *C. bergii* growth. *A. gracile* suppressed the growth of native and both alien species.

12OR.6

PSEUDO-NITZSCHIA: THE PHYSIOLOGY OF SUCCESS IN A HIGHLY STRUCTURED AND DYNAMIC PHYTOPLANKTON ECOSYSTEM

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The northern Adriatic is a very convenient ecosystem to observe phytoplankton under a variety of different conditions. Steep gradients of nutrient concentration/availability in this shallow basin allow the observation of the very same species under changing nutrient regimes on small temporal and spatial scales. The genus *Pseudo-nitzschia* is among the most abundant and most frequent diatoms in the northern Adriatic phytoplankton community over the last four decades. After studying in situ behaviour of this genus along the aforementioned gradients, we set out and isolated key species for in vitro experiments, where we analyzed their reactions to contrasting nutrient regimes. In

earlier studies, we found that alkaline phosphatase extracellular activity as well as membrane lipidremodeling are major reactions of phytoplankton to phosphate limitations. However, each species is using those functions in its own way to ensure survival and success within the phytoplankton community. Having identified phosphate limitation as a major shaping factor, we here present how harmful algae species of the genera *Pseudo-nitzschia* ensure successful competition within the phytoplankton community. Here we report data on species-specific growth rates under different nutrient regimes, phosphate uptake rates, alkaline phosphatase activity, localization and activation patterns and characteristics of alkaline phosphatase activity in four *Pseudo-nitzschia* species regularly occurring in the basin. Our results demonstrate a high interspecific variation in metabolic responses to phosphate limitation in sympatric congeneric species. The ecological significance and function appears hence to be defined on the species level and appears far from homogeneous within genera.

13. Lessons from local experiences: the Adriatic Sea

13KN.1

MACROALGAE IN THE LAGOON OF VENICE: LONG-TERM CHANGES, ALIEN TAXA AND ENVIRONMENTAL IMPACT

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The first macroalgal studies in the lagoon of Venice date back to the end of the eighteenth century. They show only taxonomic data but without mentioning either the quantitative distribution of the species or any environmental relationship. Maps of the macroalgal distribution and density have been drawn since 1980 and studies on their relationship with the environment started a few years later. Over the past 40 years over three hundreds of papers have been produced deepening the close relationship between macroalgae, trophic status and ecological status. This knowledge was the basis for the development of the Macrophyte Quality Index (MaQI), a categorical index for the evaluation of the ecological status of the Mediterranean transitional water systems. MaQI is not based on the structure and morphology of macroalgae but on the correlations of macroalgae with numerous environmental parameters, nutrients and pollutants. This index can also be applied in the presence of negligible biomass or microscopic epiphytes, according to their ecological value. In the lagoon ca. 310 taxa are present and over 80 are sensitive species that colonize only environments of good-high ecological status. Furthermore, the Venice lagoon is a hotspot of non-indigenous species (NIS). At present ca. 30 NIS colonize the lagoon accounting for ca. 32% of the total macroalgal biomass. Among them 6 species account for 98.8% of the total NIS biomass and *Gracilaria vermiculophylla* (45.3%), *Agardhiella subulata* (25.1%) and *Hypnea*

cervicornis (19.3%) are the most abundant species (89.7%). However, these NIS are not a concern for the environment. Some of them are rare species of high ecological value that increase biodiversity. Other NIS like *G. vermiculophylla* have a positive impact on the environment. This species grows under prohibitive conditions for the other macroalgae: high nutrient concentrations and very turbid waters, replacing Ulvaceae and avoiding anoxic crises.

13KN.2

LONG TERM IMPACT OF RIVERINE DISCHARGES AND NUTRIENTS LOADS IN THE NORTHERN ADRIATIC SEA

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The freshwaters discharged in the Adriatic Sea account for about 38% of the total freshwaters discharged in the Mediterranean. The northern Adriatic (NAd) rivers contribute 54% of the total freshwater discharged in the Adriatic. The Po River and other NE rivers carry relevant loads of particulate matter, dissolved organic matter and nutrients which strongly affect the trophic status of the NAd waters. Strong oscillations in the discharges occurred during the last decades which determined variations in the phytoplankton biomass abundance. The long-term discharge of Po freshwaters (1917-2017) is positively correlated with the meteorological variations at large scale (Western Mediterranean Oscillation Index) during the late spring-summer. The drought occurred in the mid 2000s determined a drastic reduction of chlorophyll concentrations in the NAd which recovered in the years 2010, 2013 and 2014. A load of P after the peak in the late 1980s revealed a decreasing trend due to the ban of P in detergents and lower use in agriculture, whereas the NO₃ load remained elevated. Accordingly, nutrient concentrations in seawater (1972-2017) monitored along the transect Rovinj-Po River mouth, the NAd evidenced

PO₄ decreasing and NO₃ increasing trends at most stations as well as an elevated NO₃/PO₄ ratio. Overall notwithstanding the recent increase of the discharges the chlorophyll a shows a long-term decreasing trend.

13OR.1

PSEUDO-NITZSCHIA SPP. IN THE NW ADRIATIC SEA: MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION, SEASONAL DISTRIBUTION AND TOXIN CONTENT

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Pseudo-nitzschia species are common representatives of the northern Adriatic diatom communities occurring throughout the year often causing intense blooms (abundances up to 10⁶ cells/l). Some species of the genus *Pseudo-nitzschia* are well known to produce domoic acid (DA) involved in Amnesic Shellfish Poisoning (ASP). The existence of cryptic and/or pseudo-cryptic species (e.g. *P. delicatissima* complex) makes identification at the species level problematic. The aim of this study is to characterize *Pseudo-nitzschia* species, during an annual cycle, in the NW Adriatic Sea. Sampling was carried out with monthly frequency in a LTER site, i.e. the coastal station of the Senigallia-Susak transect. Niskin samples were analyzed under an inverted microscope for the abundance estimation. Net samples were collected at the surface to set up cultures for molecular, ultrastructural (TEM) and toxin analyses. Seventy-five strains were established throughout the sampling period. The analysis of abundances revealed that species of both *P. delicatissima* and *P. pseudodelicatissima* complexes co-occurred during the entire investigation period with the highest abundances in May (2.8*10⁶ cells l⁻¹) and June (1.9*10⁶ cells l⁻¹), respectively. Among the *P. seriata* group, *P. pungens* and *P. fraudulenta* have been recorded mainly in winter (6*10³ and 1.4*10⁴ cells l⁻¹, respectively). The allochthonous species *P. multistriata* has been found only in winter and autumn (6*10³ cells l⁻¹). The molecular (LSU marker) and electron microscopy (TEM) analyses confirmed the presence of *P. pungens*, *P. fraudulenta*, *P. delicatissima*, *P. calliantha* and *P. mannii*. To date, no toxins were detected in 20 tested strains of *P. calliantha*, *P. mannii* and *P. delicatissima*.

13OR.2

EFFECTS OF INCREASED SEAWATER TEMPERATURE AND BENTHIC MUCILAGE FORMATION ON SHALLOW CYSTOSEIRA FORESTS OF THE WEST ISTRIAN COAST (NORTHERN ADRIATIC SEA)

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The northern Adriatic represents the northernmost biogeographic sector of the Mediterranean Sea. The sea bottom along the west Istrian Coast is mainly rocky and therefore particularly suitable for the growth of canopy-forming brown macroalgae of the genus *Cystoseira*. Comparison with historical data revealed long-term changes of *Cystoseira* species abundance. A phase of regression, occurring during the last decades of the past century, was followed by a phase of recovery. The composition of *Cystoseira* forests in the period from 2009-2013 might be considered similar to that assessed during the 1950s. Starting from 2015, a regression of *Cystoseira* forests was observed at some sites located in the central part of the west Istrian Coast. These sites were not directly threatened by anthropogenic pressure. The die-off chronology of *Cystoseira* forests was followed from spring 2015 to spring 2019. Before 2015, mixed stands of *Cystoseira compressa*, *Cystoseira barbata*, *Cystoseira crinita*, *Cystoseira foeniculacea* and *Cystoseira humilis* formed flourishing forests, which predominantly covered the rocky bottom. From February to April 2016, i.e. during the period of intensive vegetative growth of *Cystoseira* thalli at investigated sites, all deciduous branches fell off leaving perennial cauloids entirely barren. In May 2016, sparse adventitious branches developed on cauloids. However, vegetative apices were degraded impeding the regeneration of primary branches and the formation of reproductive parts. During this period epiphytes heavily overgrew the cauloids and the sites experienced a transitory sea urchin invasion. During summer 2018, surviving thalli gradually detached from the rocks initiating the final phase of the forest die-off. Unusually high temperatures during summer and the impact of benthic mucilage forming microalgal and macroalgal blooms could play a pivotal role in the decline of *Cystoseira* forests along the west Istrian Coast.

13OR.3

HIDDEN DIATOM DIVERSITY OF THE NORTHERN ADRIATIC THROUGH METABARCODING

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The northern Adriatic (NA), a shallow sub-basin of the Adriatic Sea, is under significant influence of various environmental factors that shape and determine (phytoplankton) community structures in this distinctive marine ecosystem (region). The phytoplankton community represents the base of the marine food chain and a good sensing tool of the changes in the environment. Hence, diversity of the phytoplankton has a long-term monitoring implementation in the NA. Even though, extensive knowledge of the NA phytoplankton community exists, still, some groups and species remain undetected by traditional microscopy approaches. To overcome these limitations, we implemented metabarcoding to describe diatom diversity of the NA. Sampling stations covered the entire NA region and the marine protected area Lim bay. Usage of two barcodes (18S and rbcL) and strict taxonomy assessment (99% similarity threshold) enabled detailed and reliable insights into the diatom diversity of the area studied. Common and/or abundant taxa, detected also with microscopy, were confirmed, and taxa not known so far for the area studied or taxa difficult to identify with light microscopy (pico and nano-size classes) were successfully detected through metabarcoding. A number of reads, also with high sequence similarity to reference sequences, were assigned to freshwater genera, opening further questions about their DNAs origin or species persistence in the marine environment of the NA.

13OR.4

CENTRIC DIATOMS RESPONSE TO NUTRIENT LIMITATION IN NORTHERN ADRIATIC

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As a shallow basin and under the strong influence of freshwater input (Po river), Northern Adriatic (NA) is characterized by pronounced spatiotemporal changes of nutrient concentrations over the year with unbalanced N/P ratios and also it is phosphate-limited. This represents a challenge for the phytoplankton survival and imposes adaptation of individual life strategies which results in highly structured phytoplankton community through the basin. *In situ* measurements showed that activation of enzyme alkaline phosphatase helps phytoplankton species to overcome nutrient-limited conditions. Based on the long-term data (45-year monitoring programme) on diatom diversity and related nutrient measurements, several diatom species were chosen. We isolated them from the environment, cultured in opposite nutrient conditions (phosphorus enriched and phosphorus depleted) and observed their behavior and physiological adaptations. In this paper, we focused on centric diatoms as a very important class of diatoms in NA and showed data on growth rates, localization and activation patterns of alkaline phosphatase in laboratory conditions and also showed long term *in situ* data. The combination of laboratory experiment and *in situ* data play a key role in understanding species behavior under different environmental conditions. Our results imply species level adaptation to nutrient-limited conditions. Each diatom species developed different life strategy in order to compete for nutrients and find its' own niche where it can thrive. This is of great importance to better understand and predict phytoplankton community structure in the ever-changing marine environment.

13OR.5

CHANGES IN MACROALGAL RICHNESS AND ABUNDANCE IN THE INFRALITTORAL BELT OF NORTHERN ADRIATIC SEA

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Benthic macroalgae are regularly sampled along the Slovenian coastline starting from 2006, since, in accordance with the Water Framework Directive (WFD, 2000/60/EC) requirements, they are used as a relevant biological element for the assessment of the

Ecological Status. Among macroalgae, *Cystoseira* spp. (also called canopy-forming species) are known to play an important role as ecosystems-engineers, especially in the upper-infralittoral belt. Since such long-living species follow long-term periodicity, their disappearance or even just a decrease in their abundance along the coastal rocky bottom should be considered as indicative of environmental degradation. Under key anthropogenic stressors, these species are replaced by persistent and smaller taxa, defined as turf-forming algae. These low-lying algae then form an alternative stable state that is able to inhibit recolonization by canopy-forming species. The goal of this research was an in-depth investigation of the infralittoral macroalgal cover, to search for: trends in macroalgal abundance and richness, shifts from canopy-forming to turf-forming taxa, and changes in macroalgal spatial and seasonal diversity on the infralittoral rocky bottom of the northern Adriatic Sea. At many sampling sites, significant changes in the abundance of the macroalgal cover were detected, together with fluctuations in the proportion of canopy-forming species and in the macroalgal diversity. In many Slovenian coastal areas, the resuspension of sediments along major navigation routes and due to localized dredging and construction works reach the rocky upper-infralittoral belt and, therefore, negatively affects macroalgal communities. During the 12 year-long sampling period, the sites with the highest abundance of canopy-forming taxa remained mainly those located along the Slovenian coastal belt that is still in its natural state and, thus, is very important from a nature-conservation point of view, comprising also three MPAs.

13OR.6

DIVERSITY PATTERNS AND LONG-TERM CHANGES IN THE BENTHIC MACROALGAL VEGETATION OF THE NORTHERN ADRIATIC SEA

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The northern Adriatic Sea is a shallow, semi-enclosed basin characterized by oceanographic features considerably different from the rest of the Mediterranean. The northern Adriatic was recolonized by marine biota in the last 20,000 years, after its emersion at the time of the Last Glacial Maximum. Despite of such recent hydrogeological origin, it hosts a relatively diverse seaweed flora consisting of approximately 500 species (with the highest diversity recorded for the Lagoon of Venice, over 310 species). Literature information and surveys carried out in the last two decades allow to highlight some changes in the composition of its seaweed vegetation. The most evident change is a remarkable loss of fucoid brown algae, which have decreased in distribution and abundance throughout the basin in the last 50-60 years. *Fucus virsoides*, the only endemic Mediterranean species of *Fucus*, has suffered the most dramatic decline and is now relegated to a few sites in the Lagoon of Venice, Slovenia and northern Croatia; unfortunately, this species appears now a strong candidate for extinction. Concurrently, a substantial increase in the number of introduced species took place in the last few decades, particularly in the Lagoon of Venice; many alien species are now well-established and some (*Caulerpa cylindracea*, *Gracilaria vermiculophylla*, *Agardhiella subulata*, *Hypnea cervicornis*, *Sargassum muticum*) have become locally very abundant. At the same time, recent surveys discovered a previously unappreciated diversity in some habitats, namely the offshore reefs scattered in the northernmost part of the basin (Tegnue and Trezze). At least 173 species have been reported from these biogenic concretions and molecular investigations are now unraveling a great diversity in the number of encrusting coralline algae. We suggest that future investigations on the northern Adriatic seaweed vegetation should focus on detailed assessments of species diversity in poorly-studied areas and continued monitoring of ecologically important/floristically interesting species.

14. Public outreach and citizen science

14KN.1

THE BIG SEAWEED SEARCH: SCIENCE, LESSONS AND INSPIRING THE CITIZENS

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One of the critical underpinning aspects of a citizen science project is a sense of purpose such as a robust scientific framework for generating reliable and meaningful data that will contribute to real scientific research. At the same time, the project needs to be engaging and easily achievable by anyone who wants to join in. The Big Seaweed Search serves as an excellent case study to examine these objectives. The rationale behind this project is based on three key environmental changes in the marine environment: sea temperature rise, ocean acidification and the rise of non-native species. People are invited to search the seashore around the British Isles for fourteen conspicuous and easily identifiable seaweeds whose distribution and abundance may be affected by these changes. The original project was conceived in 2006, launched in 2009 and ran until 2015. This taught us a lot about what worked well and what was less successful, notably ensuring the data can contribute to scientific research, the challenges of being able to identify seaweeds and the relatively low return rate compared to the levels of activity. This enabled us to review, revise and relaunch the project in 2016. In this talk, we will review the results of the Big Seaweed Search and explore possible ways forward for the development of citizen science projects.

14OR.1

CITIZEN SCIENCE: A SUCCESSFUL TOOL FOR MONITORING MARINE BIODIVERSITY

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The Mediterranean Sea, considered as a true hotspot of biodiversity, is currently experiencing a decline in the number of species and deterioration of habitats, as a consequence of different anthropogenic pressures, which are predicted to increase in the future. Among these pressures, the increase of human population, habitat modification and loss, pollution, coastal urbanization, overexploitation, the intentional or indirect introduction of Non-Indigenous Species (NIS, i.e. organisms introduced outside of their natural range) and climate changes (i.e. acidification and warming) have been pointed out as the major threats to biodiversity. Therefore, biodiversity monitoring and surveillance plans are strongly required in order to improve information on the distribution and change of biodiversity and/or to find and track NIS and detect early outbreaks of invasive species. In this respect, the creation of early-warning systems could be crucial. Since intensive monitoring programs are very expensive, Citizen Science, actively involving volunteers (e.g. citizen, students, fishermen, divers), could be a useful tool for gathering data, that would otherwise be impossible to collect because of limitations on time and resources. Citizen Science has grown rapidly in recent years, also thanks to mobile technologies, websites and social networks that enable an easy and cheap way to communicate, share and interchange data. Of course, in order to be used for scientific purposes and management decisions, the collected data need appropriate quality assurance measures such as validation and verification by taxonomic experts. We report on the experience of four Citizen Science activities: the Project “*Caulerpa cylindracea* – Egadi Islands”, the Project “Aliens in the sea”, the Project “Invasive Algae”, included within the “Seawatchers” platform and the Marine Forests platform. These Projects highlight how important Citizen Science is for collecting data on marine biodiversity and marine NIS and to significantly improve the efficacy of monitoring and surveillance plans.

14OR.2

FOOD FOR FISH, OXYGEN FOR ALL: BOTTOM-UP COMMUNICATION OF PHYTOPLANKTON RELEVANCE

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Despite the fundamental role they play in the aquatic environments and, as a cascade, in the provision of primary resources to human beings, the majority of the general public ignores phytoplankton existence, in our country. This communication gap stems from multiple causes, from the overall inadequacy of school programmes to the extremely weak, top-down, media-coverage gathered by aquatic ecology, especially micro-biology, in public and private broadcasts. To counteract this apparent lack of literacy, we run a series of outreach actions during the last five years. These actions included (i) public engagement with science initiatives using so-called ‘guerrilla marketing’ techniques, such as improvised microscopic sessions in the middle of feasts or bike travels through lakes and rivers, (ii) storytelling initiatives, e.g., literature and music productions relating to aquatic ecology and micro-organisms, and (iii) small outreach projects at schools of any degree. These actions targeted different age-classes. Therefore, we modulated our language and message specificity, based on the actual audience, for instance, by taking advantage of educational games (i.e., the aquatic food-web game) when speaking to children, and by using anecdotal communication (i.e., by intersecting local “ecological” tradition) when speaking to elderly people. In this paper, we present our years-long experience and show the usefulness of bottom-up strategies in communicating phytoplankton relevance to human society.

14OR.3

TEACHING IN ORDER TO LEARN ABOUT DISTRIBUTION OF INVASIVE MACROPHYTES IN THE MEDITERRANEAN SEA: USING OUTREACH AND CITIZEN SCIENCE

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The introduction of invasive alien species is a major component of global change. Invasive species have

numerous negative effects on natural resources, being the second cause (after habitat destruction) of global biodiversity loss. In addition, biological invasions pose a great threat to human beings because they can also have important negative effects on society as they impact economic, social and public health resources (e.g. fisheries, water quality, tourism, etc.). In Europe the impact of aquatic invasive species has been estimated in at least 2.2 billion euros per year, although a thorough knowledge and full of the impacts of marine invasive species is still very limited. The Mediterranean Sea is a hotspot of marine invasions, with more than 700 multicellular alien species detected, as a result of a long history of shipping, the opening of the Suez Canal and other activities. Of particular concern is the invasion of macrophytes (algae and seagrasses), as they have the potential to dramatically transform native ecosystems. The lack of selective and effective methods for the eradication of these invasive species makes early detection, monitoring and dissemination essential for adequate management and control. We have developed a project based on the design and implementation of a citizen-based system of information and monitoring of invasive seaweeds in the Spanish Mediterranean coasts. The project is based on two main pillars: 1) to develop outreach activities that educate different societal groups (schools, environmental educators, scuba diving clubs, tourism companies, etc.) on the issues of marine invasions and 2) to develop a citizen science platform (Seawatchers; <http://www.observadoresdelmar.es>) that involves all the different stakeholders in the collection of data on the distribution, abundance and ecology of invasive macrophytes. Methods, challenges and successes of this project will be discussed.

14OR.4

FIND INVASIVE SEAWEED: AN OUTDOOR GAME TO ENGAGE CHILDREN IN SCIENCE ACTIVITIES THAT DETECT MARINE BIOLOGICAL INVASION

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Invasive species threaten worldwide biodiversity. Success in facing this problem may be possible

through the engagement of younger audiences. Here, a game was designed to teach children how to recognize invasive seaweed and to encourage them to participate in marine citizen science activities. The game was first tested in a pilot study, and then it was carried out in Salinas High School (Asturias, Spain). Game-based training consisted of an explanation of invasive species, followed by the 'Find invasive seaweed' game and was evaluated with tests before and after the activity. After training, students spent 3 months looking for invasive species. Four invasive species were detected in the study area. Results showed a positive impact on knowledge gains and significant differences in marine environmental awareness and conservation actions.

14OR.5

NEED FOR INCREASED EFFORT - COULD RECREATIONAL DIVERS ENHANCE EFFICIENCY OF SEAGRASS MONITORING?

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One of the most frequent habitats in the marine protected areas in the Eastern Adriatic are *Posidonia oceanica* seagrass meadows. Volunteer

surveys led by scientists are more frequent in recent times and with them data set can be a few times greater than collect only by scientists. All volunteers need to receive basic training for research and before analysis of their data, all sets require a pass through strict main-researchers filter. During the summer of 2017 in Mljet National Park, Operation Wallacea expedition took place. During the expedition, students-volunteers have been trained according to the Operation Wallacea security standards and robust scientist framework. Research methods had to be understandable for volunteers SCUBA-divers, as groups would stay only for a week. During 7 weeks a large set of data has been collected. With a standard LIT methodology for *Posidonia* meadows research, data on meadow density (422 quadrants) and meadow consistence (119 transects) was collected. Comparison between researchers and volunteers' data showed similar results and variation among sites. It validates the global relevance of the scientist and citizen scientists sampling work. Not regarding the origin of the data, results demonstrated an increase of density compared to former research. Therefore, only multiannual research based on the exact same procedures could give dynamic trends. Volunteers' involvement was limited due to security standards, depth, equipment and staff requirements. Nonetheless, they were able to perform straight-forward tasks in a shallow and safe environment. For this expedition, the focus was on training the volunteers, as well on consistence of volunteers' data to researchers.

15. Evolutionary genetics and genomics in macro- and microalgae

15KN.1

THE EVOLUTIONARY DYNAMICS OF BROWN ALGAL SEX CHROMOSOMES

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Sexual reproduction is a pervasive phenomenon in nearly all eukaryotic lineages. In genetically controlled sexual systems, sex is determined by sex chromosomes, which have emerged independently and repeatedly during evolution. We are exploiting the exceptional richness of sexual features of the brown algae to gain novel insights into the evolution of sex determination in this group of organisms. The sex-chromosomes of several brown algal species that have diverged over a 160-MY time frame were compared, and this analysis has revealed the unique evolutionary, structural and gene expression patterns of these genomic regions compared with autosomes. Finally, using the brown alga model *Ectocarpus* sp., we have revealed how the sex chromosomes, together with autosomal loci, play a critical role not only on sex determination and differentiation but also on the regulation of other key reproductive process such as asexual reproduction via gamete parthenogenesis.

15KN.2

GENETIC DIVERSITY, PHENOTYPIC VARIATION AND THE ROLE OF COMPLEX LIFE CYCLES IN EVOLUTIONARY ADAPTATION OF BALTIC PHYTOPLANKTON POPULATIONS

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Genetic diversity is an important requirement for populations to adapt to and persist in a changing environment. In the Baltic Sea, historic bottle-neck effects and harsh environmental conditions have been suggested to reduce genetic diversity and negatively affect the capacity of populations to withstand environmental changes. Here we present empirical and experimental data on genetic structure and phenotypic diversity of two Baltic phytoplankton bloom species, *Alexandrium ostenfeldii* and *Skeletonema marinoi* and discuss these in the context of their life cycle strategies. Field surveys revealed that Baltic populations of both species are highly differentiated along spatial and temporal scales and show significant genetic diversity despite supposedly suboptimal conditions of their marginal habitats. Genetic diversity is coupled to high variation in phenotypic traits related to nutrient uptake, defense and response to environmental stressors, facilitating selection and stability of ecological functions under altered salinity conditions in experiments. The unexpected presence of high intraspecific diversity in Baltic populations of *A. ostenfeldii* and *S. marinoi* may be explained by their life cycle strategies. Both species produce dormant resting stages which accumulate in sediments. Here they form seed banks that serve as reservoirs of diversity. Sexual reproduction involved in *A. ostenfeldii* resting cyst production contributes to this diversity through recombination which can be expected to attenuate the selective effects of the bloom phase. *S. marinoi*, on the other hand, reproduces sexually when released from dormancy. This is interpreted as a preventive strategy to prepare for selection effects and respective loss of diversity happening in different phases of the bloom. The examples presented here emphasize that biology is a crucial part of adaptive responses and needs to be considered in our efforts to understand and predict phytoplankton adaptation to environmental change.

15OR.1**UNRAVELLING ASEXUAL REPRODUCTION IN TWO SYMPATRIC MORPHS OF THE AUSTRALIAN KELP (*ECKLONIA RADIATA*) USING GENOMICS**

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Evolution of asexual reproduction is considered a response to environmental conditions where it incurs less cost than sex, maintains adapted genotypes, and allows rapid proliferation into new areas. In rare circumstances, distinct asexual morphs have evolved in response to ubiquitous environmental conditions. Understanding the implications and the genetic mechanisms underlying reproduction does not only provide a fundamental understanding of life cycle evolution and regulation but is also essential to assess the vulnerability of populations to climate change. We examined morphological and genomic variation between unique co-occurring sexual and asexual morphs of the kelp *Ecklonia radiata*. Asexual morphs were morphologically distinct and less morphologically variable. We used ddRAD sequencing to identify 10,356 single nucleotide polymorphisms (SNPs) that revealed widespread clonality in the asexual morphs. These morphs had lower genetic diversity and an excess of heterozygotes relative to sexual morphs. Clustering analysis and phenology suggested that asexual morphs are still able to reproduce sexually, indicating ongoing gene flow between the two morphs. An association and Fst analysis identified candidate SNPs in coding regions segregating the morphs strongly suggesting functional differences between these morphs.

15OR.2**REVEALING POPULATION AND SELECTION DYNAMICS IN AN ARCTIC DIATOM TO ASSESS ADAPTIVE POTENTIAL TO CLIMATE CHANGE**

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Progressing climate change and concurrent fast alterations of environmental conditions pose large challenges of adaptation on organisms and ecosystems. Arctic phytoplankton, being the base of the foodweb in one of the most rapidly changing regions on the planet, is especially faced with such large transformations, but has been shown to be largely resilient towards them. In this study, we tried to tackle the question of how this resilience may be explained with a specific focus on the role and functioning of intraspecific selection and population dynamics of the diatom *Thalassiosira hyalina*. Intraspecific composition and diversity can greatly influence the productivity and resilience of a species, but selective processes driving such dynamics in phytoplankton are methodologically still difficult to resolve, especially in diverse natural contexts and at temporal resolution. Within this work, we introduce new avenues for the use of microsatellites as an efficient population genetic tool, allowing the analysis of bulk community samples in experimental and natural contexts at close temporal resolution. Through those new methodologies, we were able to resolve detailed selection dynamics in simplified diatom populations and found that their outcomes are not predictable from responses recorded in single-strain cultures. Instead, selective dynamics were reproducibly altered by the applied environmental conditions. Furthermore, in experiments with natural populations as well as in field samples from two spring blooms, our new method, microsatellite poolSeq barcoding (MPB), revealed surprising allelic stability in diverse populations over time. Sudden shifts of their allelic composition under specific environmental conditions, however, hint towards possible tipping points that limit this stability. Our results also suggest that dynamics within populations are likely driven by more complex processes than clonal dominance in highly diverse phytoplankton populations and introduce a new tool to gain a better understanding of such fundamental but often hidden mechanisms of adaptation.

15OR.3**SUBTLE DIFFERENTIATION MAINTAINS HAPLODIPLONTIC LIFE CYCLES ... UNTIL IT DOESN'T**

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Genetic models suggest haplodiplontic life cycles are transitory states on the way to diploidy or haploidy. The diversity of lineages maintaining haplodiplontic life cycles suggests they are not transitory states, but evolutionarily stable. It is only when ecological processes are considered that models predict stability. There are, however, few empirical tests of ecological niche differentiation between macroalgal ploidy or sexes. Here, we used the widespread, non-native, red seaweed *Agarophyton vermiculophyllum* (synonym: *Gracilaria vermiculophylla*), which throughout the Northern Hemisphere has been shown to undergo dramatic demographic shifts following the invasion of soft-sediment habitats. Almost all soft-substratum habitats in the non-native range are dominated by tetrasporophytes, despite the likelihood that both tetrasporophytes and gametophytes were introduced together. We sampled thalli from a site in northern California, and then subjected male gametophytes, female gametophytes, and tetrasporophytes to a factorial combination of temperature, salinity, and light level. We measured growth rates, bleaching rates, protein concentration, chlorophyll a concentration, phycobiliprotein concentration, tissue strength, C:N, dry mass, ash free dry mass, and fatty acid composition following 30 days within the treatments. The combined stress of high temperature and high salinity reduced growth and increased bleaching risk. Female gametophytes and tetrasporophytes tolerated these conditions better than male gametophytes, which showed a higher propensity to bleach overall. Less stressful environments led to higher tissue quality (e.g., increased protein, carbon, and pigment concentrations), which interacted with phase-specific differences. In the case of *A. vermiculophyllum*, the invasion of soft-substratum habitats uncouples the life cycle in which tetrasporophytic phenotypes are consistently more robust. These differences, however, are subtle and may not be captured accurately if the wrong phenotypes are investigated.

15OR.4

PHENOTYPIC PLASTICITY VERSUS GENETIC STRUCTURATION IN DINOFLAGELLATE NATURAL POPULATIONS

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Environmental fluctuations may impact microbial populations in two distinct ways. First, the organisms may adjust their phenotype to cope with environmental fluctuations. Second, environmental fluctuations may select different genotypes with variable ecological capabilities. Here, we followed the temporal population dynamics of the toxic dinoflagellate *Alexandrium minutum* *in situ* during three consecutive years. Using a metatranscriptomic dataset, we analyzed: 1. the global gene expression dynamics as a proxy for phenotypic plasticity *in situ*, and 2. the temporal genetic structure using SNPs. Gene sets showing dynamic expression correlated to environmental factor fluctuations were identified. Cellular functions traditionally studied using *in vitro* approaches, for instance, related to nutrient uptakes and metabolisms, did not display extensive expression dynamics *in situ*. Of special interest were gene sets showing higher expression in cold, low irradiance and salinity environments. One may be related to motility behaviors. The other one suggests increasing cell-to-cell interactions toward the end of the blooming period. The genetic structure was not correlated with environmental factors but would rather tend to display a pluriennial dynamic related to the species ecological success, with a temporally stable genetic structure associated with dense populations, and a temporally unstable genetic structure associated with low-density populations. This study illustrates how population structure and phenotypic plasticity may underlie microbial population dynamics.

15OR.5

POTENTIAL MECHANISMS DRIVING THE GEOGRAPHIC DISTRIBUTION PATTERN OF SECONDARY METABOLITES IN THE HIGHLY CHEMICALLY-DEFENDED RED SEAWEED *PLOCAMIMUM* "CARTILAGINEUM"

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Inter- and intraspecific interactions between organisms can either be a form of communication, environmental sensing, or defense and are often mediated by chemicals such as secondary metabolites. Sessile marine organisms such as macroalgae commonly produce chemical defenses against grazers, pathogens, as well as biofoulers. *Plocamium "cartilagineum"* is a finely branched red understory alga that is common in Antarctic macroalgal forests. It supports a very high abundance of amphipods of which most are not able to feed on the heavily chemically defended *P. "cartilagineum"* except for *Paradexamine fissicauda*. Different *P. "cartilagineum"* individuals produce differing mixtures of halogenated secondary metabolites which are referred to as chemogroups. Around Palmer Station in 2016 to 2018 a total of 16 different chemogroups were identified. The 16 chemogroups fit well into two distinct genetic groups (but not different enough to be considered different species) identified by the *cox1* and *rbcL* genes. These data suggest that chemogroups are to some extent site specific, they have some correlation with depth and that there is an underlying genetic differentiation. In order to determine the mechanisms driving the geographic pattern of secondary metabolites in *P. "cartilagineum"*, a variety of different approaches were taken. Transplant experiments were conducted to assess the role of environmental factors. Additionally, transects were run in order to describe fine-scale distribution of chemogroups and the associated population genetics.

15OR.6

POLYPHASIC APPROACH TO ADDRESS MULTIPLE SPECIES CONCEPTS FOR *HYPNEA PSEUDOMUSCIFORMIS* (GIGARTINALES, RHODOPHYTA)

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In Brazil, taxonomic studies based on molecular markers led to the description of a new species *Hypnea pseudomusciformis* to accommodate three morphologically divergent species occurring on coast, *H. musciformis*, *H. nigrescens* and *H. valentiae*. In this work, we investigate further this divergence of the phylogenetic and morphological species concepts for these species using a polyphasic approach. Specimens from two morphological variations of *H. pseudomusciformis* were collected in the field, isolated and kept in unialgal cultures (photoperiod of 14 h, 25°C and 30±10 μmol photons m⁻²s⁻¹). Crossing experiments were conducted between specimens with the typical morphology for "musciformis" and "nigrescens" to use the biological species concept to verify the molecular data. Growth rates (GRs) and photosynthesis parameters were also evaluated after a period of 28 days of culture (n=3). All crosses performed were positive evidenced by the presence of cystocarps in all-female gametophytes used. Carpospores were released and germinated in tetrasporophytic plants, which became fertile after 40 days of culture. These tetrasporophytic plants "musciformis", "nigrescens" and "F1" presented physiological differences on GRs and photosynthetic performance, which the variant "nigrescens" showed higher GRs in relation to the "musciformis". The F1 generation presented an intermediate performance between the previous two. The same patterns were observed in relation to the photosynthetic parameters: maximum quantum yield and light saturation. However, the variant "musciformis" showed higher maximum electron transport rates, while no difference was observed for the photosynthetic efficiency. The results from the crossing experiments confirm that, for Brazil, *H. nigrescens* and *H. musciformis*, belong to the same species, *H. pseudomusciformis*, which displays great phenotypic and physiological plasticity what can favor colonization of different environments and making this species very successful and widespread along the coast.

16. Algal culture collections in the -omics age

16KN.1

HOW CAN WE MAKE CULTURE COLLECTIONS SUSTAINABLE?

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Collections of algae represent an essential foundation for research and for the future bio-economy. Indeed, they are often described as heritage assets. Collections range from small personal research collections ranging from a few specific strains to several hundred strains that provide cultures to collaborators (usually at no or little cost), to large service centers that provide cultures to the wider community for a fee. Globally, algal culture collections have different business models, from full (or heavily subsidized) government support to a more corporate model where full cost recovery from culture fees are required. There is no silver bullet for making a collection sustainable, though ultimately market forces (either academic or commercial) dictate their success in the long-run. I would posit that those collections that are adaptable, have diverse funding streams, and market their unique selling points have more chance of surviving. This should not be a surprise since it is based on standard business principles. Building on his experience as Director of the National Center for Marine Algae and Microbiota (NCMA) in the US; Director of the MBA that hosts the CPR Survey plankton archive and MBA phytoplankton culture collection; as well as his role as a committee member on the CCAP science advisory board; Willie Wilson will explore different models to sustain algal culture collections.

16OR.1

THE ANACC AND AUSTRALIAN PERSPECTIVES ON DELIVERING RESEARCH IMPACT FROM AN ALGAL CULTURE COLLECTION

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The Australian National Algae Culture Collection, ANACC, manages over 1000 strains of microalgae and cyanobacteria, from freshwater, wastewater, brackish and marine, hypersaline and acidophilic environments from the tropics to the poles, since inception in the late 1960's with representatives from most established taxonomic classes. Particular species of beneficial or deleterious environmental, social and economic consequence have been studied in particular detail and as a result have a deeper biogeographic replication and coverage. In Jan 2019 ANACC accessioned its first tranche of a well-curated macroalgae collection from the University of Melbourne - a strategic move into macroalgae. The broad diversity of ANACC has necessitated a polyphasic approach to strain characterization, including morphological and life-history documentation being more recently joined by genomic typification (single and concatenated barcode genes, 16S, 18S, LSU, COI, rbcL, ITS), and in a limited number of species (cyanobacteria) by whole genome sequencing. Beyond identity, logistics and research goals dictate the priority of broader phenotype analyses that match species to their expected amplified traits; e.g. filamentous freshwater cyanobacteria screened for toxins and toxin gene expression but only opportunistically for essential polyunsaturated fatty acids. Examples will be given of core contributions by ANACC to wider CSIRO initiatives to optimize strain selection for targeted microalgae bioproducts. ANACC supplies cultures to academia and industry in over 70 countries through the Australian National Algae Supply Service (ANASS). The two core regulatory compliance issues for ANACC in the context of an Australian living biorepository open for exploitation involve Biosecurity and the Convention on Biological Diversity - Nagoya Protocol (NP). The NP is proving a very challenging article for ventures with prospective commercial interests in ANACC strains given that at the time of this abstract Australia has signed on to but not ratified Nagoya.

16OR.2

AQUATIC VIRUS CULTURE COLLECTION- AN INITIATIVE FOR THE FUTURE

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Culture collections are an essential resource for scientists globally. They provide a centralized infrastructure for the depository and continuous maintenance of different microbial species, their long-term storage, curate updated bioinformatic, phylogenetic, and physiological data on different strains, and provide services and advice to the academic and industrial scientific community. Crucially, they ensure the long-term viability of isolates, their associated physiological traits, and the preservation of their metabolic and genetic potential (all aspects, which can be lost if not regularly and properly maintained). One such resource is the Culture Collection of Algae and Protozoa (CCAP), which maintains ~3000 strains (across 600 genera) of cyanobacteria, microalgae, protozoa, and seaweed, from diverse marine, freshwater and terrestrial environments. Recently, viruses were deemed as the most abundant biological entities on the planet, reaching more than 10^6 virus-like particles in a milliliter of seawater. With the development of new methodologies for virus isolation, and the advancement of *high-throughput* sequencing technologies, we now know that viruses also encode for functionally important genes involved in lipid and carbohydrate metabolism, photosynthesis, nutrient acquisition, and the general diversification of life through horizontal gene transfer. Viruses are now also used as a tool to control certain pathogens, and as transgenic vehicles for the delivery of genes in the biotechnology sector. Moreover, the exponential increase in aquatic virology research has revealed their central role in biogeochemical cycles and climate processes. Nevertheless, although biomedically relevant pathogenic virus depositories are available, a centralized “bank” or culture collection for the growing aquatic virus research community does not exist. Here we present our vision for the development of the first of its kind aquatic virus culture collection (AVCC), showcase its preliminary integration (with chloro-, diatom-, and coccolithoviruses) into the CCAP, and highlight the benefits that this will have to the scientific community.

16OR.3

BCCM/ULC: GENOMIC RESEARCH ON POLAR CYANOBACTERIA

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The BCCM/ULC public collection of cyanobacteria aims to gather a representative portion of Polar cyanobacterial diversity from different ecological origins (microbial mats, soil crusts, cryocoliths, endoliths, etc.) and ensure their ex-situ conservation in a context of global change. These strains are available for researchers to study the biodiversity, taxonomy, evolution, adaptations to harsh environmental conditions, and genomic make-up of Polar cyanobacteria. Currently, there are 120 unicyanobacterial strains of Polar origin in the collection (catalogue: <http://bccm.belspo.be/catalogues/ulc-catalogue-search>). The collection is ISO 9001 certified for depositing and distributing strains, as part of the multi-site certification of the Belgian Co-ordinated Collections of Microorganisms (BCCM) consortium. Morphological and molecular identification (based on 16S rRNA sequences) indicate that the strains belong to the orders Chroococcales, Chroococciopsidales, Nostocales, Oscillatoriales, Pleurocapsales, and Synechococcales. This broad genotypic distribution makes the BCCM/ULC collection particularly interesting for phylogenomic studies. The first genome of an axenic Antarctic strain, *Phormidesmis priestleyi* ULC007, was sequenced. To investigate the occurrence of genes involved in the cold stress response, a selection of 42 PEGs (protein-encoding genes) linked to cold adaptation was studied in 72 cyanobacterial genomes. By comparing the genes copy numbers as a proxy of adaptation, our results underline the importance of different functions in the adaptation mechanisms to the polar environment (e.g. DNA repair, Heat shock proteins, EPS biosynthesis). We also described a metagenomic pipeline that enables the easy recovery of genomes from non-axenic cultures, tested on 17 cyanobacterial strains from the BCCM/ULC collection. In parallel, we assembled 31 co-cultivated bacteria (12 nearly complete) from the same cultures and showed that they mostly belong to Bacteroidetes and Proteobacteria.

16OR.4**ACUF: THE MICROALGAL COLLECTION AT THE UNIVERSITY OF NAPLES FEDERICO II**

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In situ conservation of aero-terrestrial microbial communities is generally not considered in conservation policy, and is particularly problematic, due to present lack of understanding of microbial ecosystems (1). To achieve affordable results, the protection of microbial diversity needs to be complemented by *ex-situ* collections, to guarantee long-term preservation and potential exploitation. Indeed, the bioprospection of microalgae from terrestrial environments spread over diverse habitats and geographical regions can lead to the formation of banks of selected strains, particularly useful for the development of biotechnological applications. The Algal Culture Collection at the University of Naples "Federico II" (ACUF) was established in 1973 by Professor Roberto Taddei and coworkers. The collection is specialized in the culture of aero-terrestrial algae, and has more than 800 strains, mostly belonging to Cyanobacteria, Rhodophyta and Chlorophyta. Special attention has been paid to Cyanidiophytina (Rhodophyta) from thermal-acidic environments: *Cyanidium*, *Galdieria* and *Cyanidioschyzon*. Presently, about 400 selected strains belonging to these latter genera are maintained in culture and have shown a relevant cryptic genotypic diversity (2). Most ACUF strains have been extensively used for taxonomic, physiological, and eco-toxicological studies; instead others resulted promising as potential candidates for biodiesel production, biological sequestration of CO₂ and wastewater treatment also cultivated in high tech photobioreactors. Lastly, some selected strains are under pilot development for the production of long-chain polyunsaturated fatty acids, protein, sugars and secondary metabolites (such as pigments and vitamins) to be used as high-value ingredients for food, cosmetic, nutraceutical and pharmaceutical industries. Metadata and active links to scientific information

on the ACUF strains are also planned as ICT activities on the ACUF website (ww.acuf.net) for better usability by the scientific community and industrial stakeholders.

16OR.5**THE NAGOYA PROTOCOL ON ACCESS AND BENEFIT SHARING: IMPLEMENTATION STATUS IN ALGAL BIOLOGICAL RESOURCE CENTRES**

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The Nagoya Protocol (NP) on Access and Benefit Sharing (ABS) is a legally binding agreement supplementing the Convention on Biological Diversity. Its objective is the fair and equitable sharing of benefits arising from the utilization of genetic resources, thereby contributing to the conservation and sustainable use of biodiversity.

Today, 116 countries are a party to Nagoya Protocol. National ABS focal points and competent national authorities are defined in 172 resp. 105 countries. The ABS Clearing House (<https://absch.cbd.int/>) provides information about these institutions and national legislations plus a huge variety of administrative documents. But only two national ABS procedures are documented and many countries still did not implement ABS legislation yet.. It is often not clear what is needed to legally access, use and transfer genetic resources and how to proof due diligence. Definitions of genetic resources and utilization vary. However, collectors, depositors, researchers and bioprospectors must ensure legal clarity in how they can (or cannot) use genetic resources. Algal culture collections are specialized biological resource centers (BRCs) and main stakeholders to follow NP legislation and raise awareness. They actively participate in national and international developments on the implementation of ABS legislation and Best Practice Guidelines. Decisions to the current discussion on defining basic research as utilization and on expanding NP scope to include digital sequence information will effect not only algal BRCs but also most fields of phycological research. European Union Regulation 511/2014 implemented NP legislation for EU users including a 'register of collections'. Such registered

collections prove to meet a number of specific, nationally defined criteria and their users automatically demonstrate due diligence when sourcing material (e.g. living algal cultures or DNA). Nevertheless,

registration has practical implications for many BRCs that currently exceed their scope and resources. Best practices of NP implementation in algal BRCs and their use will be discussed.

Workshop 1: Measuring photosynthesis with light: application and new insights in active fluorescence techniques

WIKN.1

PULSE AMPLITUDE MODULATED (PAM) FLUOROMETRY: THE MEASUREMENT PRINCIPLE, THE PARAMETERS, THE RELATION TO ELECTRON TRANSPORT RATES AND ITS USE TO STUDY NON-PHOTOCHEMICAL QUENCHING (NPQ)

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In this introductory talk, the major parameters which can be obtained by PAM analyses will be explained. What do they mean? What can they tell and what not? How are they measured correctly? Besides this general methodological part, new data regarding the understanding of NPQ (Non-Photochemical Quenching) processes in diatoms will be presented. By having created mutants with varying NPQ capacities in *Phaeodactylum tricornutum*, we could study in-depth their NPQ properties by PAM. Combining these data with data obtained by Fast Repetition Rate Fluorometry (FRRF) enabled us to get deep mechanistic insights into NPQ and to specify the exact subtypes of NPQ. These results enlarge our understanding of the molecular regulation of thermal dissipation in diatoms substantially.

WIKN.2

APPLICATION OF FAST REPETITION RATE FLUOROMETRY (FRRF) IN PRIMARY PRODUCTIVITY STUDIES: A TUTORIAL

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Phytoplankton primary production is a sensitive ecosystem indicator and in coastal systems driven by local drivers which can be very different in adjacent systems. As phytoplankton primary production delivers the organic matter which fuels aquatic foodwebs, knowledge of primary production (PP) is essential to understand ecosystem change and functioning. Unfortunately, PP measurements are on the decline, most likely because the ¹⁴C-method, i.e. the most commonly method to measure PP is not easily applicable in monitoring programs and health and safety regulations make it more and more difficult to use this technique. In this tutorial we suggest an alternative to measure primary production, based on the application of FRRF. This technique measures the quantum efficiency of photosynthesis of PSII, and can be used to derive primary production and can be used to derive information about the physiological status of the phytoplankton. It is now possible to measure concentrations of reaction center-II and to measure the absorption properties per unit volume. In this lecture we will describe the principles of the technique and show how recent developments make it possible to derive absolute rates of photosynthetic

electron transport (ETR) and how these can be converted to rates of C-fixation. We will also discuss the assumptions and uncertainties of this method and how they may be related to the physiological status of the algae. Despite the remaining uncertainties, which are not only due to conversion factors from ETR to C-fixation, but also to inherent uncertainties in the C14-method, we suggest that this method can be generally adopted as a standardized method to measure primary production, allowing better comparison between PP estimates.

WIKN.3

COMBINING FLUORESCENCE AND ABSORPTION SPECTROSCOPY TO INVESTIGATE CYCLIC ELECTRON FLOW AROUND PHOTOSYSTEM I IN VARIOUS MICROALGAE

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It is commonly assumed that in plants and green algae, cyclic electron flow (CEF) around photosystem I (PSI) plays a crucial role in optimizing photosynthesis. CEF increases the electrochemical proton gradient and is therefore believed to have two main roles, (i) regulating the photosynthetic control and non-photochemical quenching in photosystem II (PSII), and (ii) providing the extra ATP required for carbon fixation. However, several decades of research did not provide a clear-cut answer about the mechanism, extent, regulation and conservation of CEF among different photosynthetic clades. This is mostly due to the absence of a consensus method to estimate this flow in physiological conditions. The most widespread approach compares the quantum yield of PSII, through fluorescence techniques, and the one of PSI, through the absorption changes associated with variations of the redox state of its special pair, P₇₀₀. But we could show that the latter method tends to underestimate the quantum yield of PSI, leading to aberrant conclusions regarding CEF. We propose an alternative method based on the electrochromic shift of photosynthetic pigments, to test for the presence of CEF and, where appropriate, describe the relationship between LEF and CEF. We could highlight 3 behaviors: in some photosynthetic species, CEF is not

occurring under steady state illumination (that is the case in the dinoflagellate *Amphidinium carterae*). In the ones where CEF exists, it can be independent of LEF (e.g. the dinoflagellate *Symbiodinium*) or dependent on LEF (e.g. the green alga *Chlamydomonas reinhardtii*). This method can be used to explore how CEF depends on physiological and environmental conditions.

WIKN.4

THE FLUORESCENCE-BASED ESTIMATIONS OF GROSS AND NET PRIMARY PRODUCTIVITY IN A MARINE DIATOM - THE EFFECTS OF DYNAMIC NUTRIENT STRESS

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We have studied the effect of NO³⁻ limitation on the electron transfer and carbon fixation and allocation in marine diatom *Chaetoceros muelleri*. In order to simulate nutrient changes occurring under natural conditions, we manipulated the concentration of the nitrate from fully replete to severely limiting. This created a dynamic continuum of physiological states of balanced and unbalanced growth that were sampled on daily basis for suite of photosynthetic, cellular and growth parameters. The primary productivity was quantified using several methods: by the FRR-based algorithm, by the coincident C and O₂ (MIMS) isotopes based estimations of gross (GPP) and net (NPP) primary productivity. Overall, we observed strong correlation between the fluorescence-based estimates of ETR and the simultaneously measured carbon fixation during nutrient replete and the dynamic, non-steady states of the onset of nitrate limitation, but uncoupling between these proxies for GPP under late phases of nutrient limitation. The relationship between the optical cross section determined spectrophotometrically and the optical cross-section of PSII (aLHII) remained linear, apart from a short period during the transfer from moderate to strong-N-limitation, suggesting that the K_a remained constant during most of the unbalanced growth phases. We observed much larger variability between the ETR and the “standard” method to estimate GPP using 30-min ¹⁴C incubations: both coincided for the nutrient replete state, but increasingly deviated (3-4x) with the progress of nutrient stress. The deviation is well correlated with the instantaneous growth rate. Interestingly, the growth efficiency, determined from

the ratio of NPP to ETR was low (12%) under replete conditions but doubled under moderate nitrate stress. Under extreme nutrient stress, when the growth effectively stopped, the growth efficiency has also declined dramatically. To our knowledge, this is the first study to reveal the dynamics of photosynthetic electron transfer rates and carbon storage efficiency in diatoms under variable nutrient conditions.

WIKN.5

PHOTOPHYSIOLOGICAL STRATEGIES TO IRON LIMITATION AND HIGH LIGHT DIFFER BETWEEN TWO ANTARCTIC KEY PHYTOPLANKTON SPECIES

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As iron (Fe) and light availability strongly influence phytoplankton species distribution in low Fe-waters, we investigated the combined effects of increasing light (20, 200 and 500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) in conjunction with different Fe (0.4 and 2 nM Fe) availability on the physiology of two ecologically relevant phytoplankton species in the Southern Ocean, *Chaetoceros debilis* (Bacillariophyceae) and *Phaeocystis antarctica* (Haptophyceae). Combining Fast Repetition Rate fluorometry, elemental composition and pigment analyses with cell-based modelling, new insights on the photophysiological strategies of the two tested species were gained. Fe-deficient cells of *P. antarctica* displayed similar high growth rates at all irradiances. In comparison, Fe-deplete *C. debilis* cells grew much slower under low and medium irradiance and were unable to grow at the highest irradiance. Interestingly, Fe-deficient *C. debilis* cells were better protected against short-term excessive irradiances than *P. antarctica*. Next to similar electron transfer rates, Fe-deplete *C. debilis* cells displayed faster re-oxidation of the primary electron acceptor Q_a , indicating operation of a putative plastid plastoquinol terminal oxidase, known to create a proton gradient in the thylakoid lumen. In line with this, high xanthophyll activity was shown by its high cellular diadinoxanthin content along with very high NPQ activities. Such strategy was especially efficient after short-term exposure to high irradiance, as seen by the high potential of F_v/F_m recovery (~70-80%), pointing towards a high tolerance of *C. debilis* to short-term high light stress. This tolerance was,

however, counteracted by strongly lowered growth and particulate organic carbon production rates of the diatom relative to the prymnesiophyte. The prymnesiophyte also possessed high photoprotective capabilities, with strong alternative electron cycling activities (electron cycling around photosystem I, Mehler and/or photorespiration) being more important than xanthophyll cycling. Overall, our results suggest that *P. antarctica* could outcompete *C. debilis* in Fe-deficient waters at all light regimes.

WIOR.1

EFFECTS OF PS I FLUORESCENCE AND CHLOROPLAST RELOCATION ON CHLOROPHYLL FLUORESCENCE

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Higher plant studies have demonstrated that PS I fluorescence contributes up to 50% to total F_o fluorescence, and that chloroplast movements can reduce the F_o level by 30%. Quantifications of these factors and their effects on saturation pulse analyses will be reviewed. PAM chlorophyll measurements to study circadian chloroplast movements in algae (e.g., *Ulva lactuca*, *Acetabularia mediterranea*) will be discussed.

WIOR.2

BLINDED BY THE LIGHT: DARKNESS REVEALS THE COMPLEXITY OF ARCTIC DIATOMS PHOTOADAPTATIVE STRATEGY

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Pulse Amplitude Modulated (PAM) fluorometry is a widely-used technique in studies of photosynthesis with various light treatments. It allows to distinguish the amount of light energy channeled into an electrochemical gradient from the excess, potentially harmful, one dissipated as heat via the non-photochemical quenching (NPQ). Applying PAM fluorometry once growing or stressful irradiance is turned off, can also give tremendous insights on subtler physiological reactions ongoing in darkness or under recovering

low light. Diatoms of the Arctic Ocean exploit an extreme light environment, controlled by the seasonal dynamics of the snow/ice cover coupled with extreme photoperiod contrasts. Therefore, adaptations to frequent light fluctuations and prolonged periods of obscurity are to be expected, but few studies have been conducted on the matter. To address this question, we grew five arctic diatoms species in the lab, representative of distinct niches over the seasonal continuum of the light environment: from the snow-covered dimly lit bottom ice to the autumnal stratified waters. Using different light exposures, dark incubations and inhibitors, we tried to determine how highly contrasted light environments could have shaped diverse photoadaptive strategies. As usually observed in diatoms, all species relied heavily on fast responding NPQ to shift between light harvesting and photoprotective states. However, clear discrepancies were revealed when comparing their long-term relaxation kinetics following light exposures. Sustained NPQ, chlororespiration and photosystem repair cycle were differentially expressed across species and photoacclimations. Through structural modulations of photosystem II and plastoquinone redox reactions, these mechanisms influence fluorescence emissions and were monitored by PAM fluorometry. These unusual photoadaptive features, alongside strong «classical» NPQ, could explain how arctic diatoms tread the thin line between meeting metabolic demands and mitigating photo-oxidative stress in a harsh light environment at freezing temperatures.

WIOR.3

THE ROLE OF SUSTAINED PHOTOPROTECTIVE NON-PHOTOCHEMICAL QUENCHING IN MICROALGAE

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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 and high irradiance suggests that they use XC related NPQ to dissipate light

energy absorbed in excess under constant light. Under balanced growth at moderate and high irradiance, NPQ is often sustained (hours-days kinetics relaxation) and the quenching efficiency (QE) of Dt or Z is very low. We investigated the characteristics of NPQ in the haptophyte *Tisochrysis lutea* and in the Arctic diatom *Thalassiosira gravida* under various growth conditions (Low Light, high light, N starvation, N limitation in a chemostat, P starvation, L/D cycles). Both species showed sustained NPQ (NPQs) at moderate/high irradiance, which is very difficult to quantify using PAM fluorometry. When cells were suddenly exposed to an increasing light gradient, a new NPQ (NPQd) developed due to additional Dt synthesized from Dd de-epoxidation (as proven by DTT effect). The QE of the newly synthesized Dt was much higher than the QE of the preexisting Dt. NPQd extent was inversely proportional to NPQs. The potential role of NPQs in the photophysiological plasticity of microalgae, especially under adverse growth conditions, is discussed.

WIOR.4

ENHANCEMENT OF PHOTOSYNTHESIS IN SYNECHOCOCCUS BACILLARIS BY SPONGE-DERIVED AGELADINE A: A THEORETICAL PERSPECTIVE

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Marine sponges harbor many symbionts, which constitute a large amount of the sponge's dry weight. Symbionts of sponges, such as the cyanobacterium *Synechococcus*, likely serve a role similar to that of the gut microbiome in mammals. The sponge *Agelas* sp. produces a brominated pyrrole alkaloid, Ageladine A, which accumulates in acidic cellular compartments and has previously been used as a pH sensitive, fluorescent dye. Ageladine A, may therefore also accumulate in the acidic thylakoid space of photosynthetic cells. The ecophysiological role of Ageladine A in the sponge biome, however, is still unresolved. This study shows that Ageladine A acts as an additional light harvesting molecule for photosynthesis of the symbionts. A joint modeling and experimental

approach revealed that Ageladine A facilitates photosynthesis of *Synechococcus*, when the cells are exposed to UV light. Due to the presence of Ageladine A, photosynthetic production of O₂ increased 2.54 and 3.1-fold, in the experiments and the model,

respectively. While the absorbance of Ageladine A is in the UV range, it fluoresces blue, matching the blue absorbance of chlorophyll a. This study, for the first time, presents a type of symbiosis in which light is the major currency of species interaction.

Workshop 2: Handling large genomic datasets

W2KN.1

ASSEMBLY AND ANALYSIS OF THE LAMINARIA DIGITATA GENOME AND TRANSCRIPTOME

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Kelp species can form forests which provide important benefits for other species. We set out to analyze the genome of *Laminaria digitata* and to assess the variability within and between populations. The genome is estimated to be around 700 MB long preventing the complete assembly from short reads only. Thus, we not only prepared DNA for short Illumina based reads but also high molecular weight DNA for Nanopore sequencing from female and male gametophytes. Hybrid assembly of raw reads resulted in ~700 Mb scaffolds indicating near full coverage of the haploid genome. Transcript data were used to enhance the reliability of gene prediction. I will discuss challenges of genome assembly of such large genomes and characterize it in comparison to already published brown algae genomes.

W2KN.2

ECO-EVOLUTIONARY DRIVERS AFFECT LIFE CYCLE CHANGES AND MICROEVOLUTION OF THE EMILIANIA/GEPHYROCAPSA SPECIES COMPLEX

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Emiliana huxleyi has become the numerically dominant or co-dominant coccolithophore in essentially all of the surface ocean since its first appearance only 291000 years ago. In contrast, its closest living relatives in the genus *Gephyrocapsa* exhibit much more restricted distributions. We have conducted a set of

phylogenomic, comparative transcriptomic/genomic, population genomic, and physiological experiments using the *Emiliana/Gephyrocapsa* complex to understand this ongoing evolutionary story. Phylogenomic studies reveal with involving all five species in the *Emiliana/Gephyrocapsa* complex show *E. huxleyi* forming a monophyletic clade only in nuclear multi-gene coalescent phylogenies, not in organellar phylogenies. The incongruence of the chloroplast, mitochondrial, and nuclear phylogenies suggests incomplete lineage sorting in large populations and a history of introgressive hybridization. Of the SNPs which distinguish nuclear clades within the species complex, between 9% and 25% occur in protein-coding sequences, with average Ka/Ks ratios ranging from 1.26 to 2.62, suggesting positive selection on a small number of genes is driving diversification in these organisms. We also found that strains living in lower latitude regions further from the coast where the abundance of their conspecifics remains low to have a tendency to lose genes specific for the haploid motile phase. RadSeq studies of genetics nearby populations with and without the tendency to lose 1N strains suggested that loss of haploid genes might correspond to obligate parthenogenesis. Finally, we are exploring whether such genomic changes correlate with physiological differences in response to nutrient starvation. In summary, a history of several evolutionary processes is recorded in *E. huxleyi* and its close relatives, providing a model for understanding the evolutionary processes involved in phytoplankton sympatric speciation, range expansion, and adaptation to new environments.

W2KN.3

LEVERAGING SEQUENCE AND EXPRESSION INFORMATION FROM DE NOVO TRANSCRIPTOMES; STRATEGIES AND CASE STUDIES FROM THE BROWN ALGAE

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For the vast majority of algae, genome sequences are unavailable, while access to the resources and

expertise to generate them are in many cases still limited. Given this, transcriptome sequencing (RNAseq) will remain an important and accessible tool to generate sequence information for coding genes, and to allow expression analysis in experimental studies. Focusing on brown algae (a group for which some genomes are becoming available), we will discuss strategies for generating *de novo* transcriptomes using short-read RNAseq data. Raw *de novo* transcriptomes tend to be large and complex. Therefore, post-assembly curation strategies based on re-mapping, transcript quality assessment and clustering are useful to reduce redundancy and complexity. Additional challenges arise due to potential contamination of field-collected, non-axenic or non-unialgal samples, which complicate gene expression and sequence-based (phylogenetics, hybridization) applications. Finally, specific challenges are associated with brown algal genome organization that can result in “polycistronic” transcripts that must be addressed prior to downstream analysis. To illustrate some of these issues and solutions to deal with them, we draw from two recent case studies involving kelp transcriptomes (Phaeophyceae, Laminariales). The first is an analysis of gametogenesis in *Saccharina latissima*, to identify sex-specific and -independent processes underlying the gametogenic developmental programs of male and female gametophytes. The second is an analysis of homoploid hybrid sporophytes derived from crosses between *Laminaria digitata* and *L. pallida*; two hybridizing kelp species with different thermal optima.

W2KN.4

EXPANSION OF PHYCOBILISOME LINKER GENE FAMILIES IN A MESOPHILIC RED ALGA, *PORPHYRODIUM PURPUREUM*

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The common ancestor of the ecologically and economically important red algae (Rhodophyta) underwent massive genome reduction over 1 billion years ago, whereby ca. 25% of the gene inventory shared with their sister green algae and plants was lost. This was followed by the split of Rhodophyta into the species-poor extremophilic Cyanidiophytina and the highly successful (>7000 species) mesophilic lineages.

It is unknown which innovations explain the diversification of mesophiles given their highly reduced gene inventory. To address this important issue, we combined a significantly improved long-read assembly from the unicellular red alga *Porphyridium purpureum* with a variety of other algal genomes to reconstruct ancient endosymbiotic gene transfer (EGT) and gene duplication events. We report evidence of ancient EGTs associated with the plastid translocons and core photosynthetic machinery that likely played a fundamental role in plastid establishment. More significant to the major focus of our study is the finding of extensive duplications and diversification of nuclear-encoded phycobilisome linker proteins that play central roles in stabilizing light-harvesting functions. We interpret these latter results as evidence that the origin of complex phycobilisome linker proteins in mesophilic red algae allowed them to adapt and radiate under different light regimes. This finding addresses a key question about red algal evolution and more broadly, illuminates how a major group of eukaryotes “escaped” a depauperate gene inventory to conquer a large swath of the near-shore marine ecosystem.

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USING GENOME SCALE METABOLIC NETWORKS TO STUDY ALGAL BIOLOGY AND EVOLUTION

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Large-scale genomic and metagenomic projects today can comprise hundreds or even thousands of different genomes. Metabolic networks can provide a useful tool to filter this wealth of information and extract biologically and evolutionarily relevant features of the metabolism of the different species. Here we present a suite of tools (AuReMe, Compare) to automatically generate robust metabolic models from heterogenous genomic data and to compare and analyze them. The tools were designed to simultaneously deal with several tens of eukaryote and hundreds of prokaryote genomes. By including gene orthology information in comparisons of metabolic models, we minimize the impact of qualitative

differences between the genomes and their annotations. We then give two examples of how these tools can be applied to answer biological questions. First, we discuss a global comparison of 39 metabolic networks of representative multi- and unicellular algae from the green-, red-, and stramenopile lineages to identify metabolic specificities of selected groups and species. These analyses highlight, for instance, differences between uni- and multicellular stramenopiles with respect to oxylipin- and cell wall metabolism. They also pinpoint subtle differences at the metabolic scale between closely related brown algal species. In a second example we show how

metabolic networks can be used to interpret a complex metagenomic and meta-transcriptomic dataset of the brown alga *Ectocarpus subulatus* interacting with different bacterial communities. The aim was to identify bacterial metabolic functions likely related to the capacity of the alga to grow in fresh-water and metabolic maps were reconstructed for different bacterial communities identified in response to this stress. These analyses suggest the importance of maintaining selenoamino acid metabolism, but also underline the production of bacterial quorum sensing molecules in cultures that are maladapted to fresh water.