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## Keynote and Oral Papers

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## Keynote and Oral Papers

### 1. Algal diversity and species delimitation: new tools, new insights

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#### 1KN.1

##### HOW COMPLEMENTARY BARCODING AND POPULATION GENETICS ANALYSES CAN HELP SOLVE TAXONOMIC QUESTIONS AT SHORT PHYLOGENETIC DISTANCES: THE EXAMPLE OF THE BROWN ALGA *PYLAIELLA LITTORALIS*

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The great phenotypic variability and the lack of diagnostic characters in the genus *Pylaiella* render the systematic study of this genus problematic. In the present study, we investigated the diversity of *Pylaiella littoralis* (Linnaeus) Kjellman along the Brittany (France) coast using a DNA barcoding multilocus approach with mitochondrial (*cox1*, *nad1* and *atp9*) and chloroplastic (*rbcL* and *atpB*) markers associated with a population genetics approach using 10 microsatellite markers. In addition, spatio-temporal sampling was conducted along the Brittany coast. Mitochondrial sequence results revealed the occurrence of two cryptic species, with a minimum of 2.4% divergence between them. Microsatellite genotypic data recovered three well supported clusters matching the two mtDNA clusters of *Pylaiella*. While gene flow is limited between mitochondrial clusters, occurrence of genetic admixtures in some populations suggests that reproductive isolation is not complete. Our study highlights how the combination of phylogenetic and population genetics approaches can help determine algal diversity and study boundaries between closely related species.

#### 1KN.2

##### GENERATING THE DIVERSITY - UNCOVERING THE SPECIATION MECHANISMS IN FRESHWATER AND TERRESTRIAL MICROALGAE

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Species are one of the fundamental units of biology, comparable to genes or cells. Understanding the general patterns and processes of speciation can facilitate the formulation and testing of hypotheses in the most important questions facing biology today, including the fit of organisms to their environment and the dynamics and patterns of organismal diversity. Though eukaryotic microorganisms are extremely numerous, diverse and essential in global ecosystem functioning, they are largely understudied by evolutionary biologists compared with multicellular organisms. In part owing to their small sizes and difficulty in culturing, our knowledge of their diversity and evolutionary processes is considerably limited. In particular, very little is known about speciation mechanisms generating the diversity of microalgae. In this talk, I will present several examples of speciation mechanisms detected in freshwater and terrestrial algae, including limited dispersal, natural polyploidization, and ecological divergence. In general, climatic- and habitat-niche differentiation seems to represent the most important force that drives the origin of new species. Accordingly, despite the cosmopolitan distribution of many eukaryotic microorganisms, local fine-scale structuring of ecologically distinct genotypes may represent the most plausible explanation for their extremely high species richness.

#### 1KN.3

##### HIGH-CONTENT FLUORESCENCE MICROSCOPY APPLIED TO MARINE PROTISTS ECOLOGY

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The mainstream tool in marine ecosystems biology is massive DNA sequencing, allowing detailed inventory of microbial environmental genetic diversity. However, the large majority of meta-omics data, in particular in eukaryotic size-fractions, are not yet assigned to any described organisms. This greatly limits our understanding of the ecosystemic and biogeochemical functions of the genetically unveiled diversity. Linking environmental genes and genomes to organismal phenotypes and morphology is arguably one of the next big challenge in ecosystems ecology. Few available in-flow imaging technologies (FlowCytoBot, CytoSense, ImageStream, FlowCAM . . .) propose a valuable imaging rate that may match Next Generation Sequencing sensitivity. Unfortunately, the trade-off between speed and image quality significantly impacts the images information content and limits analyses to relatively narrow organismal size ranges. We developed a novel strategy – we call *e-HCFM* for environmental High-Content Fluorescence Microscopy – directly inspired from high-throughput quantitative fluorescence microscopy for cell biology. Our protocol includes planktonic cells fixation/multi-staining/mounting/automated imaging and taxonomic annotation. The fluorescent labeling strategy aims at making visible all eukaryotic cells by highlighting a few key features: nuclei, compartmentalization (i.e. membranes), chloroplast and exo-skeleton. The use of Confocal Laser Scanning Microscopy (CLSM) allows 3D-imaging analysis of the wide range of cell size and morphologies displayed by planktonic protists. It significantly improves the quality and quantity of measurements extracted from each imaged cells. *e-HCFM* may bring critical information on eukaryotic cells shape, volumes, and organelles, contributing to both automated taxonomic identification and recognition of key biological and biogeochemical features at the single-cell level, including life-cycle stages and potential symbioses *sensu lato*. A case-study based on samples from the *Tara Oceans* expedition is presented. The primary version *e-HCFM* described herein can be used as a backbone for several staining protocols

targeting diverse biological function, in order to tackle all sorts of ecological questions.

## 1OR.1

### GAPS TO FILL WHEN ANALYZING FRESHWATER DIATOM DIVERSITY WITH DNA BARCODING – NOTES FROM A BOREAL REGION

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The idea of using DNA metabarcoding as a more reliable and most of all cost-effective tool for environmental assessment based on freshwater benthic diatom biodiversity has enchanted stakeholders now asking when these tools will be available for routine usage. However, traditional methods using diatom taxa data generated by microscopical identification have a good rumor to be very effective tools in the assessment of water quality status, and we should be careful to just exchange methods. Before molecular data can become a standard for assessing algal diversity in environmental assessment, we need to compare the results of both to analyze advantages and drawbacks of each method. Here, we present a first analysis of a setup of ecologically very different streams of Sweden in a quantitative comparison of DNA metabarcoding (rbcL barcode) and microscopical counts. We used using next-generation sequencing, a dedicated bioinformatics pipeline and a curated DNA reference library. Many of the dominating diatom species of the boreal region were missing in the database, which complicated the methods comparison. Still, even when taxa were represented in the reference database, we found quite a large number of taxa being detected by only one of the methods. We hypothesize that at least a part of this difference depends on the choice of the barcoding gap, because we found that the relative abundance of certain taxa decreased or increased depending on which gap was used for the reads assignment. We suggest that the barcoding gap should be adapted to the clade in question, as it is not homogenous among different diatom groups. Our perspective is to achieve more complete and correct taxa lists for both

methods as another step for the routine usage of meta-barcoding methods in the future.

### 1OR.2

#### **GENDER STUDIES IN SEAWEEEDS - EXPRESSION AND EVOLUTIONARY RATES OF SEX-BIASED GENES IN FUCOID BROWN ALGAE**

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Sexually dimorphic males and females share a largely common genetic background, leading to the suggestion that gene expression is the major determinant of sexual phenotype. Sex-biased genes controlling reproductive functions are candidates having roles in lineage divergence and speciation processes. We have analysed sex-biased expression in several members of the family Fucaceae by comparing the transcriptomes of reproductive tissues, and used sequence information to study patterns of adaptive evolution in a phylogenetic framework. Comparative transcriptomics of male and female reproductive tissue against the vegetative background in *Fucus vesiculosus* revealed many more male-biased (MBG) than female-biased genes (FBG); 1,127 and 174 transcripts, respectively. Expression analysis of orthologues showed that MBG were more consistently sex-biased across different species than FBG. This might be related both to male specialization (e.g., the presence of flagella uniquely in sperm cells), and to female functional pleiotropy. Specialization was illustrated by the fact that over 60 % of MBG in *F. vesiculosus* were uniquely-expressed in male reproductive tissue. These male-unique genes (MUG) showed distinctive evolutionary patterns, including reduced codon-bias compared with female-, or non-biased genes (i.e., reduced GC3 content, and higher effective number of codons), suggesting relaxation of selective pressure for translational efficiency and/or accuracy. Analysis of 439 genes with codon-based branch-site models of adaptive evolution showed that the number of sites under positive selection was greatest in the MUG group, followed by MBG, FBG and non-biased genes. Together, our results suggest that tissue-specific expression is linked to reduced selective and/or pleiotropic constraint, which in turn may drive accelerated rates of adaptive divergence.

### 1OR.3

#### **PHYLOGENETIC AND BIOGEOGRAPHICAL PATTERNS OF ALLOPOLYPLOID SPECIATION IN AN INTERTIDAL FUCOID SEAWEED ASSEMBLAGE**

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Modern molecular tools are contributing to a renewed interest in the roles of hybridization and polyploidy in evolution. These processes combine in allopolyploid speciation, best known from plants and a few vertebrate lineages. Additional models across the tree of life may provide new insights underlying the factors leading to allopolyploid formation, establishment and evolutionary success. We investigated the evolutionary history of *Hesperophycus/Pelvetiopsis*, a brown algal (Stramenopiles) seaweed complex, screening a diverse panel of populations of *Hesperophycus californicus* and *Pelvetiopsis limitata* sensu lato using a variety of nuclear/organelle sequencing/typing markers. Four species were recovered, including *P. arborescens* (taxonomically unclear previously) and a new unrecognized species. Gene paralogy, cyto-nuclear conflict, fixed heterozygosity and allele states strongly supported the allopolyploid nature of two of these entities and the male contribution of *P. arborescens* to both. *P. arborescens* has an extremely reduced and non-overlapping geographical distribution compared with the most recent (and possibly still expanding) allopolyploid taxa. Conversely, the older allopolyploid is presently the most widespread taxa, although its maternal ancestral is probably extinct. Our results provide evidence for ecological divergence allowing allopolyploids to locally co-exist with diploid parental species, which may even be outperformed in the long term.

### 1OR.4

#### **ELUCIDATING UNRESOLVED INVASION HISTORY WITH GENOME-WIDE SEQUENCING APPROACH: THE CASE OF THE GLOBAL INVADER *SARGASSUM MUTICUM***

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The brown seaweed *Sargassum muticum* is one of the most emblematic non-indigenous seaweed species. Native to Asia, it has successfully invaded the North-Eastern Pacific coasts since the 1940s and the



North-Eastern Atlantic since the 1970s. Introduction history in these two areas remained unresolved because introduced populations were found to be genetically monomorphic. For instance, a previous study showed that all 1200 individuals, from 46 introduced American and European populations, shared the exact same multi-locus genotype at 14 microsatellites. To understand this pattern, and elucidate the invasion history of *S. muticum*, we developed a genotyping-by-sequencing method (ddRadseq), for the first time in an invasive seaweed. Large scale libraries were successfully sequenced for 303 individuals from 21 populations (Europe: 9, North-West America: 7 and Japan: 5). Conversely to previous studies, genetic polymorphism was detected in the introduced populations with 2026 SNPs ( $H_e = 0.15$  and  $0.12$  in American and European areas respectively). Nevertheless, much larger diversity was found in the native range and, as expected, severe founder events were evidenced in the introduction ranges. A decreasing number of polymorphic loci was observed between the native range, NW America and Europe (1259, 455 and 232 respectively) as well as strong genetic similarities between America and Europe; both supporting predictions of a secondary introduction in Europe from America where *S. muticum* was introduced earlier. Genetic structure was observed within each introduction range. In Europe, the Mediterranean and Portuguese populations differed significantly and in America, populations from the Puget Sound were highly differentiated from those of southern California. While the link between genetic diversity and invasion success has often been highlighted in marine systems, our results document a success story of a global invasion with limited genetic polymorphism. More generally, they confirm the relevance of genome-wide strategies to investigate the genetic diversity of weakly polymorphic algal species.

## 1OR.5

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

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The subclass Corallinophycidae encompasses four orders of benthic red algae unique by the presence of calcite. While the order Rhodogorgonales is a species-poor and mainly a tropical group of gelatinous algae with calcification limited to calcite husks, the orders Sporolithales, Hapalidiales and Corallinales are diverse and widely distributed, characterized by having calcified walls and highly variable morphology. Despite recent clarification of phylogenetic relationships, the origin and evolution of this group is still controversial. Based on the presence of distinctive anatomical features, the literature dated back the first fossil records in the early Cretaceous (140 Mya); however, the presence of ancient extinct taxa resembling coralline red algae could suggest an earlier origin back to the Neoproterozoic (*ca.* 600 Mya). In this study we will apply a molecular evolution approach integrating comprehensive molecular data and fossil records toward estimating the degree of genetic divergence among lineages and their time of evolutionary divergence as well as the age of their most recent common ancestor. This approach will involve 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 encompassing different genera will be compiled in order to obtain the most accurate timeline for calibrating the Corallinophycidae phylogeny. Diagnostic characters employed for the distinction at the suprageneric level pertaining to the three phases of their life cycle will be also analysed in order to select characters, which have relevant signal in light of the evolution of coralline algae inferred from our phylogeny.

## 1OR.6

### THE PATHOGENS OF BROWN ALGAE *ANISOLPIDIUM ECTOCARPII* AND *ANISOLPIDIUM ROSENINGEI* DEFINE A NEW CLASS OF MARINE ANTERIORLY UNICILIATE OOMYCETES

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Despite their abundance in the field, and their suspected role in regulating the abundance of their host population, hyphochytrid pathogens of brown algae have been hardly studied. Using laboratory cultures, we document here the life cycle of *Anisolpidium ectocarpii*, a pathogen of *Ectocarpus* and other filamentous brown algae, and present preliminary observations on *Anisolpidium rosenvingei*. Consistent with earlier reports, the zoospores of both species have a single anterior flagellum, which justified the placement of *Anisolpidium* amongst the Hyphochytriales (Hyphochytridiomycota). Unexpectedly, nuclear (SSU rRNA) and mitochondrial (cox 1, cox2) markers regroup *A. rosenvingei*, *A. ectocarpii* and other marine environmental sequences into a hitherto unrecognised monophyletic clade within the Oomycetes (Oomycota), most closely related to the Olpidiopsidiales and Haliphthorales. The *Anisolpidium* genus is therefore entirely distinct from the Hyphochytriales and represents the first unquestionable instance of an anteriorly uniciliate oomycete. We also show that *A. ectocarpii* can complete its infection cycle in a broad selection of species from various brown algal orders, suggesting that species delimitation within the genus *Anisolpidium* should not merely be based on the identity of the algal host, as is presently the case. Finally, a working hypothesis is generated in an attempt to establish a new criterion for the separation of hyphochytrids from oomycetes, based on the point of zoospore cleavage.

### 1OR.7

#### A MOLECULAR APPROACH TO *NITZSCHIA*: ESTABLISHING A FRAMEWORK FOR CLASSIFYING A HIGHLY DIVERSE AND TAXONOMICALLY DIFFICULT DIATOM GROUP

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*Nitzschia* Hassall is now probably the largest single genus of diatoms (perhaps 1000 species), split in >10 sections (Cleve & Grunow 1880). It is also remarkable for being highly diverse: with respect to its ecology (e.g. common and often abundant in freshwater, brackish and marine habitats; some species recognised as bioindicators of organic or metal pollution, etc), physiology (e.g. phototrophs, heterotrophs, mixotrophs), life cycle (e.g. homo- and heterothallic, automictic, avoiding size reduction). However, *Nitzschia* is also notorious for its taxonomic difficulty: several species have very few diagnostic characters in the LM. Furthermore, based on morphology several genera have been separated from *Nitzschia* (e.g. *Tryblionella*, *Psammodictyon*, *Simonsenia*) but their interrelationships are still unclear. We assembled a four-gene data set (nuclear LSU and SSU, plastid *rbcL* and *psbC*) of several species of *Nitzschia* and related genera. Analysis shows that *Nitzschia* and even some of its sections are non-monophyletic as previously suggested by some single or two-gene phylogenies. Our results also pin point characters that have been conservative during evolution (e.g. ultrastructure of striae or systems of strips found in the auxospore wall), whereas automixis has evolved at least twice independently in *Nitzschia* sect. *Lanceolatae*.

### 1OR.8

#### THE ORIGIN OF DIVERSITY IN GREEN ALGAE

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Mutations are the main drivers of diversity and speciation. They generate the genetic diversity natural selection acts upon. Exploring the process of mutation is fundamental for our understanding the diversification and the evolution of eukaryotes. The spontaneous

mutation rate is currently available for a few model organisms including the freshwater green alga, *Chlamydomonas reinhardtii*. We will present the results of mutation accumulation experiments in five strains of unicellular haploid marine green algae; 4 Mamiellophyceae (*Ostreococcus tauri*, *O. mediterraneus*, *Micromonas pusilla*, *Bathycoccus prasinosi*) and one Trebouxiophyceae (*Picochlorum* spp.). These phytoplanktonic organisms are at the base of the food web in coastal oceanic areas. Their genome sizes from 13 Mb to 22 Mb and their GC composition from 46 to 65% makes them ideal species to investigate the interplay between genome architecture and mutation rates. The mutation rate is assessed by whole genome re-sequencing of one mother line and an average of 30 lines maintained for 5000 generations per species.

### 1OR.9

#### CHEMISTRY MEETS BIOSYSTEMATICS: PARALLEL STUDIES ON THE DIVERSITY OF THE *LAURENCIA* COMPLEX (RHODOMELACEAE, RHODOPHYTA) IN SOUTH AFRICA

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The *Laurencia* complex is one of the most studied algal groups with regard to secondary metabolites. A taxonomic study in South Africa, using morphology and anatomy as well as sequencing of the *rbcL* marker, has raised the diversity from an initial 10 species of *Laurencia*, to 14 species (including 5 described as new), and produced the first records of *Palisada*, *Chondrophycus* and *Laurenciella*. This has increased the known South African species to 19, plus 6 potential taxa to be described. Isolation and detailed characterization of secondary metabolites of 8 of these species, including two of the new species, produced 31 compounds, some of them original. Algoane, which was previously only reported from a sea-hare, proved to be a unique marker compound isolated from *Laurencia*

*natalensis*. Broad predictions of a specimen's phylogeny, based on representatives of its secondary metabolome, proved viable. Related species possess similar chemical profiles, e.g. *L. complanata*, *L. sodwaniensis* sp. nov. and *L. multiclavata* sp. nov. produced similar metabolites to their sister species as inferred by the *rbcL* phylogeny. In addition, a <sup>1</sup>H NMR profiling study on the crude organic extracts of various *Laurencia* species generated distinctive, reproducible spectra, showing the value of NMR spectroscopy as a rudimentary species discernment tool.

### 1OR.10

#### SPECIES DIVERSITY AND MOLECULAR PHYLOGENY OF THE CRUSTOSE CORALLINE ALGAE (CORALLINALES, RHODOPHYTA) FROM THE WARM WATER WESTERN PACIFIC OCEAN WITH AN EMPHASIS ON SPECIES FROM TAIWAN

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Crustose coralline algae (CCA) are important marine producers and play a critical role in marine ecosystem including many reef-building species. CCA are classified into two red algal orders - the Corallinales and Sporolithales, which are characterized by having calcareous deposits within the cell walls and all reproductive structures developing in conceptacles or pits. Previous taxonomic records of CCA in Taiwan were solely based on morphological observations. Our present study is the first attempt to apply for a molecular tool for documenting the species diversity and inferring their intra- and interspecific relationships based on *psbA* and SSU sequence analyses. In this study, more than 100 CCA collections were collected and sequenced from Taiwan and the neighbouring islands. Seven natural assemblages (= genera) and more than 18 subclades (= species) were revealed based on two gene phylogenetic analyses. In particular, one large assemblage contains some undescribed species positioned within the *Lithothamnion/Mesophyllum/Phymatolithon* complex. We will further test the significance of the morphological characters currently used for separating *Mesophyllum* and *Phymatolithon* and related genera. More samplings and phylogenetic analyses will be conducted to unravel the species diversity and their biogeographic relationships in the warm water western Pacific Ocean in order to understand their evolutionary histories.



**1OR.11****LITHOPHYLLUM CONGESTUM  
(CORALLINALES, RHODOPHYTA) WHAT  
ARE YOU AND WHAT AREN'T YOU?  
RECEIVING SOME INNER SIGNALS FROM  
DNA AND MORPHO-ANATOMY TO CLARIFY  
OUR UNDERSTANDING ON THE SPECIES**

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*Lithophyllum congestum* (subfamily Lithophylloideae) is reported as a common species forming algal ridges around the Caribbean and it has been recorded as south as Brazil. This species has been recently considered a heterotypic synonym of *L. stictaeforme*, changing our circumscription of both species. In this study we analysed material of *Lithophyllum congestum* housed at National Museum of Natural History (US) from the Caribbean, including countries as Cuba, Jamaica, Puerto Rico, Virgin Islands, Caribbean British Islands and Martinique. In addition, we also studied the type material housed at The NTNU University Museum at Norwegian University of Science and Technology (TRH). We aimed to elucidate the taxonomic status of *Lithophyllum congestum* complemented with data on morphology and anatomy, molecular diversity and distribution. In addition, we compared our results obtained for *L. congestum* with Mediterranean collections identified as *L. stictaeforme* and also with records of *L. kotschyianum*, a species widely recorded from coral reefs. Our results show that i) coralline herbarium material, even after 148 years in the case of type material or 37-49 years in the case of more recent Caribbean

collections treated in a wide range of drying and storage conditions still provide short but informative sequences (*rbcl* 300 bp, *psbA* 600 bp); ii) Mediterranean collections identified as *Lithophyllum stictaeforme* does not match the genetic information of *L. congestum* obtained from type and herbarium material, suggesting that the synonymization is incorrect; iii) *PsbA* sequences of *Lithophyllum congestum* from the Caribbean are identical and possibly conspecific with specimens from Madagascar, Australia and Japan, but also with some records identified as *L. kotschyianum*, changing previous schemes on its distribution range. This case study on *Lithophyllum congestum* aims to provide new strategies and decisions for studies of coralline algae species and diversity, focusing on molecular markers, sampling strategy and morphological analyses.

**1OR.12****RIPPLES OF THE PAST: HOW MUCH  
ENDEMISM IS THERE IN SEAWEEDS?**

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Since the application of molecular techniques in the identification and systematics of the seaweeds (red, green and brown macroalgae), it has become increasingly clear that there is considerably more diversity than hitherto recognised that is not necessarily reflected in the morphology of these organisms. One of the consequences of the recognition of 'cryptic diversity' is a fundamental shift in defining species concepts, with profound implications for understanding distribution, rarity and endemism. The definition of what constitutes an endemic, i.e. a species which is confined to a certain region, is reasonable, but asking how much endemism really exists in the seaweeds poses a number of questions. Using examples from my work on red, green and brown seaweeds, I will explore the concept of endemism in relation to taxonomic hierarchy, patterns of distribution, reproduction and dispersal, and consider whether there are any general principles we can learn from these organisms. I will also consider how vital knowledge of endemism is in conservation policy and management of biodiversity.

**1OR.13****DEPAUPERATE ENVIRONMENTS: CRADLE  
OF SPECIATION OR EVOLUTIONARY DEAD  
ENDS?**

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In terms of marine biodiversity, the Baltic Sea is a depauperated mass of water relatively isolated from the North Atlantic with a stable salinity gradient that poses a strong selective regime to any marine species willing to colonize it. The established populations of marine species that colonized it after the last glaciation are at their ecological and geographical limits. However, previous research in the Baltic Sea has shown adaptations to this environment that have produced an extremely rapid speciation event in *Fucus* seaweeds. Here, we used a population genomics approach with RAD-Seq to revisit this case and we found that what was previously believed to be a single case of speciation might be a cryptic radiation instead. Despite no apparent physical barriers, the permanent salinity and temperature regimes, combined with the water circulation patterns in this region may effectively isolate populations even at very short geographical distances, creating quick and large genetic differences among demes in many areas along the Baltic.

#### 1OR.14

##### **MATCHING NAMES AND CLADES IN THE BROWN ALGAL GENUS *LOBOPHORA* (DICTYOTALES, PHAEOPHYCEAE): AN EFFORT TO INTEGRATE TYPE SPECIMENS IN MODERN TAXONOMY**

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The use of gene sequence data has profoundly altered our view on algal diversity on every taxonomic level. Most spectacularly, sequence data unveiled the existence of massive cryptic or pseudocryptic diversity at the species level. Cryptic diversity also makes linking DNA-based lineages to existing taxa exceedingly difficult, which results in a growing tendency to move from a formal algal taxonomy to a more informal system whereby clade-, specimen- or strain-based identifiers are used to communicate biological information. Counteracting the emergence of parallel taxonomies would require a better integration of historical collections into modern taxonomic research. Here we explore the feasibility of linking taxonomic names, often described decades before DNA-based species identification became the norm in phycology, to clades in modern phylogenies. The brown algal genus *Lobophora* forms an excellent test case to explore the feasibility of such efforts. Recent studies have demonstrated that the genus is far more diverse than traditionally assumed. For decades only a handful of species were recognized of which *L. variegata* (J.V.Lamour.) Womersley ex E.C.Oliveira was by far the most commonly reported. DNA-based species delineation methodologies, however, indicate that there may be at least over one 120 species. We reevaluated the identity of 17 taxa suspected to be representative of *Lobophora* species by attempting DNA amplification of historical herbarium material as well as specimens recently collected from the type locality (epitypes). Our results indicate that exclusive reliance on information preserved in type specimens is problematic. For several species, either the type could not be traced or we did not get permission to extract DNA from types. Epitype material proved a more successful way forward, but this route often comes with a considerable degree of uncertainty, especially in tropical regions where the degree of sympatry among *Lobophora* lineages is sometimes considerable.

#### 1OR.15

##### **BIODIVERSITY IN THE PLANKTONIC DIATOM FAMILY CHAETOCEROTACEAE**

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Chaetocerotaceae is one of the most diverse marine planktonic diatom families. Its two genera, *Chaetoceros* and *Bacteriastrium* currently include over ca 230 and 15 taxonomically accepted species, respectively. We set out to assess its diversity at the LTER station MareChiara in the Gulf of Naples (GoN), Mediterranean Sea, at Roscoff, along the French Atlantic coast, and at Las Cruces in an upwelling zone on the central Chilean coast. Colony and spore morphology, frustule ultrastructure as well as nuclear LSU and SSU rDNA sequences have been gathered from ca 240 monoclonal strains. Results showed 55 genetically distinct species, many of these being new to science. Phylogenies inferred from the aligned sequences revealed several major clades of morphologically similar species. SSU sequences in one of these clades contained up to 6 introns of ca. 140 base pairs each. In addition, many of the internal standard sequencing primers showed mismatches in critical positions. V4 regions in the SSU were used as taxonomic references and blasted against an environmental V4-sequence dataset gathered from 40 plankton samples taken over three years at the LTER station in the GoN. Results provide an unprecedented picture of the diversity and seasonal cycle in this species-rich family.

## 1OR.16

### MICROALGAE COMMUNITIES IN ANTARCTIC SOILS: CHANGES ALONG SOIL DEVELOPMENTAL STAGES AND TESTING FOR GEOGRAPHICAL DISTRIBUTION

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Goals of this study are a precise assessment of the species diversity of Antarctic soil algae and to assess their functional traits by investigating defined sampling plots along different temporal developmental stages of soil after glacier retreat (chronosequences). Selected Antarctic soil algal species will be tested for possible endemism. The nuclear-encoded ITS2 rRNA region is targeted to provide sufficient resolution for species identification and comparisons with counterparts from other continents. The ITS2 analyses were facilitated by easier PCR amplification when compared to the plastid-encoded *rbcL* gene and by employing tools initially developed for fungal sequences, e.g. for annotation. To enrich the clone libraries with sequences from algae, PCR primers to preferentially amplify rDNAs for certain algal groups were found inevitable because more general PCR primer combinations underestimated the algal diversity. The composition of the soil algae assemblages varied considerably along the studied samples, which represented different soil ages. A large number of algal clones could be identified with high similarities using BLAST sequence comparisons, especially for the Klebsormidiophyceae, some Trebouxiophyceae and Chlorophyceae. For *Klebsormidium*, *Interfilum* and *Desmococcus* sequences were recovered with very high ITS2 similarities (99 and 100%) with those of isolates from temperate regions. A considerable number of Ulvophyceae, difficult to recover from soils using cultures, was revealed. For other algal groups, e.g. the Xanthophyceae, species identification was hampered because of a limited number of available reference sequences. Full reference sequences need to be established from cultures, i.e. from culture collections or establishing new isolates. The cloning/sequencing of long amplicons including conservative regions (5.8S, parts of SSU and LSU) enables the assignment of algal clones where no closest ITS2 references are available as well as chimera check. This approach also provides the reference sequences for the development of group-specific primers for the shorter amplicons required for pyrosequencing.

## 1OR.17

### ULVALES ON GERMAN BALTIC AND NORTH SEA COASTS: ABOUT CRYPTIC, ALIEN AND LOST SPECIES

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Green macroalgae of the order Ulvales are abundant and often dominant in European marine and brackish shallow water environments and thus ecologically important. Some of their representatives are characteristic foulers. Therefore Ulvales often get introduced into new ecosystems where they have a high potential of affecting algal diversity and ecosystem services. However, their morphology is often extremely variable, which largely hampered their taxonomic identification until recently. Cryptic or alien species are often not recognized, while different phenotypes of the same species are in many cases regarded as distinct taxa. Here we report the results of a survey on Ulvales biodiversity in the Baltic and North Sea areas of the German state of Schleswig-Holstein and adjacent areas that was based upon analyses of more than 700 samples with different genetic barcoding markers (*tufA*, *rbcL*, ITS). Molecular analyses based on sequences of *rbcL*, ITS and *tufA* genes combined with morphological analyses revealed so far the presence of 20 different species including 4 cryptic and 7 newly introduced taxa (thereof one potentially invasive) that were not known from the area yet. Further, several species that were until now estimated to occur in high abundance are seemingly very rare or even absent.

#### 1OR.18

##### OLD AND NEW PROBLEMS IN THE CIRCUMSCRIPTION OF MEDITERRANEAN SPECIES OF *LITHOPHYLLUM* (CORALLINALES, RHODOPHYTA)

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Coralline algae are among the most common seaweeds in benthic habitats worldwide, as well as one of the most species-diverse groups. Unfortunately, they are also among the most problematic in terms of species circumscription and characterization. Their taxonomic

history has been plagued by problems of cryptic diversity, phenotypic plasticity and taxonomic misinterpretations related to the importance of some morphological characters. Key tasks in current coralline taxonomy are to clarify the extent of genetic variation of individual species and link type specimens with molecular phylogenetic clades. We examined three Mediterranean species of *Lithophyllum* (*L. byssoides*, *L. incrustans*, *L. stictaeforme*) using a combination of molecular markers (*cox2,3* spacer and *psbA*, *rbcL* and 18S rDNA genes). The results unraveled different scenarios for the three species. Mediterranean *L. byssoides* represents a well-defined entity that should be separated at species level from Atlantic populations reported under the same name. *Cox2,3* and *psbA* data showed an unexpectedly high variation, suggesting limited dispersal in populations of this species. *Lithophyllum incrustans* is the type species of the genus *Lithophyllum* and its characterization is therefore critically important. A partial *rbcL* sequence obtained from the type material allowed to bridge the gap between molecular sequences and name-bearing type. In light of this result we concluded that *L. incrustans* is common in subtidal habitats and may easily form rhodoliths. Intertidal crusts reported as *L. incrustans* both in the Mediterranean and in Atlantic Europe probably do not belong to this species. *Lithophyllum stictaeforme*, as currently circumscribed on morphological basis, represents a complex of cryptic species for which a taxonomic and nomenclatural reassessment is necessary. Taken together, our results indicate that the genetic diversity of Mediterranean coralline algae is probably much higher than currently believed and sequences from type specimens will be essential to unambiguously identify many species.

#### 1OR.19

##### TAXONOMIC REVISION OF *PAPENFUSSIELLA* SPECIES IN THE NORTHERN HEMISPHERE

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Molecular phylogeny of *Papenfussiella* species using mitochondrial *cox3* and chloroplast *rbcL-rbcS* DNA sequences revealed considerable species diversity in the genus. Although only *P. kuromo* is currently recognized in Japan, the specimens referable to *Papenfussiella* collected in Japan formed four distinct

clades (clade-1, -2, -3 and -4) with high statistical support. Specimens of clade-1 corresponded to *P. kuromo*, because the sequences as well as the morphology agreed well with those of the lectotype specimen. Specimens corresponding to clade-2 were similar to *P. kuromo* in gross morphology, but were distinguished anatomically by the presence of a subcortical layer. Clade-3 consisted of the syntype specimens of *P. kuromo* f. *densa* Inagaki, having narrower thalli with denser branches, and we propose to treat this taxon as an independent species. Clade-4 consisted of specimens from Eastern Hokkaido. They formed a clade with *P. callitricha* but were genetically distinct. In addition, preliminary study of Southern Hemisphere *Papenfussiella* species also suggested the occurrence of several more cryptic species in the genus.

#### 1OR.20

#### REVISION OF THE SECTION *MACROSPORA* KUMANO OF THE GENUS *BATRACHOSPERMUM* (RHODOPHYTA, BATRACHOSPERMALES)

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A revision of the section *Macrospora* was carried out based on morphological analysis (including new col-

lections and type specimens) and molecular data (using the plastid RUBISCO gene *rbcL* and the mitochondrial barcode region of the gene *cox1*). Only one species (*B. macrosporum* Montagne) was recognized in the section based on the currently applied morphological diagnostic characters: carpogonial branches long ( $\geq 6$  cells and  $\geq 65 \mu\text{m}$  in length); distal involucrel filaments short (1 cell), forming a crown around the carpogonium; large carposporangia ( $\geq 21 \times 19 \mu\text{m}$ ). Two species were proposed as synonyms: *B. equisetifolium* Montagne and *B. hypogynum* Kumano & Ratasabapathy. Genetic variation within this species was highly variable: 0–69 base pairs (bp), 0–10.4% for *rbcL* and 0–85 bp, 0–6.6% for *cox1*. Five groups were recognized within *B. macrosporum* applying two single-locus DNA-based species delimitation techniques. All groups have clear-cut genetic limits and some have a defined geographic distribution. The five groups were recognized as cryptic species but they are not formally proposed as distinct species. These groups occurred in tropical and subtropical regions of the world and were particularly widespread in North and South America. Molecular phylogenetic analyses showed *Macrospora* as a well-supported clade within the Batrachospermales. An additional morphological character (post-fertilization carpogonial branches with enlarged pit connections) was evidenced as a new morphological character to support the circumscription of the section. We propose the raising of the section to the genus level.



## 2. Shedding new light on photosynthesis and its role in global biogeochemistry

### 2KN.1

#### MIXOTROPHS IN AQUATIC ECOSYSTEMS: PRIMARY PRODUCERS OR CONSUMERS?

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Microalgae are well recognized as key players in the carbon cycle by contributing about half of global primary production. However, next to carbon fixation many microalgae are also capable of heterotrophic nutrition by feeding on other microorganisms. These mixotrophs are increasingly recognized as important components of microbial food webs. Due to their nutritional versatility the net contribution of mixotrophs to primary production and ecosystem respiration is variable and dependent on environmental conditions. While physiological experiments using the chrysophytes *Ochromonas* spp. indicate a tight integration of the photosynthetic and heterotrophic metabolism with a tendency towards photoheterotrophy, both components can be affected differentially by environmental factors. For example increasing temperatures cause a shift in the nutritional strategy towards heterotrophy. Increasing rates of prey ingestion have also been observed in a climate change scenario for a natural phytoplankton community from a temperate lake. Such shifts in the nutrition of mixotrophs will affect microbial population dynamics and modulate the cycling of both carbon and nutrients.

### 2KN.2

#### BIODIVERSITY OF GAPDH REGULATION IN MICROALGAE

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Diatoms are a widespread and ecologically important group of heterokont algae that contribute ca. 20% to global productivity. Previous work has shown that regulation of their key Calvin cycle enzymes differs from that of the Plantae, and that in crude extracts, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), an enzyme involved in this CO<sub>2</sub> assimilation pathway can be inhibited by NADPH under oxidizing conditions. The freshwater diatom, *Asterionella formosa*, was studied using enzyme kinetics, chromatography, surface plasmon resonance, mass spectrometry and sequence analysis to determine the mechanism behind this GAPDH inhibition. In this diatom, GAPDH interacted with ferredoxin–nicotinamide adenine dinucleotide phosphate (NADP) reductase (FNR) from the primary phase of photosynthesis, and the small chloroplast protein, CP12. Sequences of co-purified GAPDH and FNR were highly homologous with published sequences. However, the widespread ternary complex among GAPDH, phosphoribulokinase (PRK) and CP12 was absent. Activity measurements under oxidizing conditions showed that NADPH can inhibit GAPDH-CP12 in the presence of FNR, explaining the earlier observed inhibition within crude extracts. The absence of the ternary GAPDH-CP12-PRK complex may be due to the absence of two cysteine residues at position C243 and 249 (numbered from the alga, *Chlamydomonas reinhardtii* on diatom PRK. Diatom plastids have a distinctive metabolism, including the lack of the oxidative pentose phosphate pathway, and so cannot produce NADPH in the dark. The observed down-regulation of GAPDH in the dark may allow NADPH to be rerouted towards other reductive processes contributing to their ecological success. However this GAPDH regulation is absent in other diatoms such as *Thalassiosira pseudonana*, opening questions about evolution and ecology.

### 2KN.3

#### SYSTEMS-WIDE ANALYSIS OF ACCLIMATION RESPONSES TO LONG-TERM HEAT STRESS AND RECOVERY IN THE PHOTOSYNTHETIC MODEL ORGANISM *CHLAMYDOMONAS REINHARDTII*

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Occasions of extreme heat stress HS may severely reduce plant growth. Therefore, a comprehensive understanding of HS as a consequence of global warming is of significant interest for agriculture and aquaculture industry. In a system-wide analysis, using the unicellular green alga *Chlamydomonas reinhardtii* as a model system, acclimation responses to long-term HS were studied in a time-resolved manner and under controlled conditions. For this, we shifted cells from 25 to 42°C for 24 h and back to 25°C for  $\geq 8$ h and monitored changes in abundances of proteins, metabolites and lipids and changes of many cytological and physiological parameters. The data indicate that acclimation of *Chlamydomonas* to long-term HS is coordinated and implemented as sub-responses that can be temporally and functionally connected and were therefore termed “response elements”. These are executed in order to prevent irreversible damage and to maintain cellular homeostasis, thereby allowing cells to acclimate to HS and to survive. Five response elements could be distinguished: (i) the arresting of DNA replication and cell division; (ii) catabolism of larger molecules to generate compounds with roles in stress protection; (iii) accumulation of molecular chaperones to restore protein homeostasis together with compatible solutes; (iv) redirection of photosynthetic energy and reducing power from the Calvin cycle to the de novo synthesis of saturated fatty acids to replace polyunsaturated ones in membrane lipids, which are deposited in lipid bodies; and (v) when sinks for photosynthetic energy and reducing power are depleted, resumption of Calvin cycle activity associated with increased photorespiration, accumulation of reactive oxygen species scavengers, and throttling of linear electron flow by antenna uncoupling. During recovery from HS, cells appear to focus on processes allowing rapid resumption of growth rather than restoring pre-HS conditions.

## 2OR.1

### USING FAST REPETITION RATE FLUOROMETRY TO ESTIMATE PSII ELECTRON FLUX PER UNIT VOLUME: A PURELY OPTICAL METHOD FOR ESTIMATING GPP BY PHYTOPLANKTON?

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Obtaining accurate estimates of GPP by phytoplankton in highly dynamic environments, such as complex coastal seas, continues to present a major challenge to oceanographers. Although Fast Repetition Rate Fluorometry (FRRF) has long been used for *in situ* estimation of GPP, the requirement for an independent method to determine the concentration of functional photosystem II reaction centres ([RCII]) has limited the application of this method. A new approach to the analysis of FRRF data has allowed for estimation of [RCII] from dark FRR data alone (Oxborough et al. 2012). The same approach to the analysis of FRR data has also led to development of an ‘absorption algorithm’, which can be used to generate wavelength-specific PSII absorption coefficients (aLHII values, with units of m<sup>-1</sup>) from dark FRR measurements. Importantly, the product of aLHII and PSII photochemical efficiency under ambient light (which can also be estimated from FRR data, through the Fq’/Fm’ parameter) provides an estimate of GPP which does not require determination of [RCII] to estimate GPP and provides a greatly improved signal to noise ratio under high photon irradiance. Since all parameters required for application of the absorption algorithm are derived from FRR data, this approach to the estimation of GPP can be applied on wide spatial and temporal scales and can realistically be considered as a viable alternative to photosynthetron-based measurements of <sup>14</sup>C uptake. This talk will cover recent developments and outstanding issues related to application of the absorption algorithm, including the processing of data to minimise the error generated by fluorescence from sources other than functional RCII (baseline fluorescence, F<sub>0</sub>-b).

**2OR.2****HIGH RESOLUTION FRRF MEASUREMENTS TO MEASURE NET AND GROSS PRIMARY PRODUCTION**

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In this presentation, we will show how we can use Fast Repetition Rate Fluorometry (FRRF) as an alternative means to measure primary production, and how we can automate the technique, so that it can be used autonomously on ships or on fixed stations. Recent developments now allow to measure absolute rates of electron transport and obtain concentrations of PSII reaction centres. We will briefly explain this, give examples of measurement and show high resolution time measurements obtained at several locations. Comparisons with <sup>14</sup>C-measurements demonstrates the annual variability in electron requirements for C-fixation at several sampling stations in Dutch marine waters and we will show how we can obtain daily and annual rates of primary production. Lastly, we will show, using cultures, how the electron requirement for C-fixation changes going from gross to net photosynthesis.

**2OR.3****NITROGEN AVAILABILITY DRIVES VARIABILITY OF THE ELECTRON REQUIREMENT FOR CARBON FIXATION IN COASTAL PHYTOPLANKTON COMMUNITIES**

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Active chlorophyll fluorescence induction techniques have the potential to revolutionise our understanding of the nature and extent with which primary productivity

(PP) varies within marine ecosystems. However, major uncertainty still exists in how PP estimates determined using fluorometry (absolute electron transfer rate, ETR) scale to corresponding rates of carbon (C)-uptake. To address this gap, studies have simultaneously measured ETR and C-uptake to derive the so-called “electron requirement for carbon fixation” ( $e:C$ ), and commonly observed that this conversion factor varies according to the dominant phytoplankton taxa present under steady-state growth. However, no study has yet resolved to what extent  $e:C$  potentially varies when systems are perturbed, particularly when nutrient availability impacts upon phytoplankton physiology and/or community structure. Therefore, we performed fully multi-factorial macronutrient bioassays (N, P and Si) upon natural phytoplankton communities collected from a coastal reference station (Port Hacking, NSW) near the Eastern Australian Current during summer, when macronutrients are known to be limiting. Our results consistently demonstrated that  $e:C$  responded strongly to N addition, either in isolation or in combination with P and/or Si. In the control bottles,  $e:C$  typically ranged between 10-12 mol e [mol C]<sup>-1</sup>, values which are higher than expected for a large range of phytoplankton when under steady-state growth, but decreased threefold (3-4 mol e [mol C]<sup>-1</sup>) and close to the theoretical minimum for the N treatment. This influence of N for two of the bioassays (December, February), was strongly driven by a taxonomic shift (also reflected by an increase in the >10µm size-fraction), whilst the third bioassay (January) was conducted immediately following a transient bloom, resulting in a strong physiological response of the existing population. Our unique approach therefore demonstrates that  $e:C$  can be influenced by both taxonomic and physiological adjustments to phytoplankton communities, but both driven by an underlying limitation of N availability.

**2OR.4****EFFECTS OF INCREASED CO<sub>2</sub> AND IRON AVAILABILITY ON THE MECHANISMS OF CARBON ASSIMILATION AND CALCIFICATION DURING A BLOOM OF THE COCCOLITHOPHORE *EMILIANA HUXLEYI***

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A mesocosm experiment was carried out in the Raunefjord (Bergen, Norway) to investigate the



interactive effects of increased CO<sub>2</sub> and iron availability during a bloom of the coccolithophore *Emiliania huxleyi*. The seawater carbonate system of half of the mesocosms was manipulated to achieve CO<sub>2</sub> levels corresponding to predicted future conditions (900 ppmV) as compared to present ambient conditions (390 ppmV). Dissolved Fe concentrations were also manipulated by addition of the siderophore desferrioxamine B (DFB) promoting an increase in dissolved iron (dFe). The experiment consisted of a multifactorial design with combinations of both levels (high and ambient) of CO<sub>2</sub> and dFe. Primary production rates were measured by in-vitro incubations based on <sup>14</sup>C-fixation and particulate calcium was measured by wavelength dispersive X-ray fluorescence (WXRF). Primary productivity (PP) was enhanced as a direct consequence of the *E. huxleyi* bloom and the Ca/POC ratio presented the highest values and a significant linear regression in the same treatment as PP during the bloom. PIC/POC ratios supported such data. Maximum molar [POC]:[PON] ratios were observed at the end of the experiment; thus the treatments had significant effects on C:N ratios. The isotope disequilibrium assay was used to determine the relative fraction of HCO<sub>3</sub><sup>-</sup> and CO<sub>2</sub> uptake. The fraction of HCO<sub>3</sub><sup>-</sup> uptake obtained for all treatments indicated a high rate within the community over time. The highest rate of extracellular CO<sub>2</sub>: HCO<sub>3</sub><sup>-</sup> interconversion ( $\alpha$ ), as a measure of extracellular carbonic anhydrase activity (eCA) changed as the community shifted. The relevance of these results within the global change scenario will be discussed.

## 2OR.5

### THE ROLE OF CARBON CONCENTRATING MECHANISMS IN MACROALGAL RESPONSES TO OCEAN ACIDIFICATION

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The majority of fleshy macroalgae, across all groups, contain carbon-concentrating mechanisms (CCMs) that facilitate use of dissolved inorganic carbon. Rising atmospheric CO<sub>2</sub> concentrations and the resulting shift in seawater carbonate chemistry and ocean acidification (OA) may have varying effects on algae depending on their mode of inorganic carbon concentration, if any, and its regulation. Energetic costs of these carbon uptake mechanisms may change with rising pCO<sub>2</sub> and may explain the difference in effects of OA on growth and photosynthesis. We investigated the relative activity of CCMs in the green alga *Ulva lactuca* and the red alga

*Plocamium cartilagineum* across a range of atmospheric CO<sub>2</sub> concentrations (385-1000  $\mu$ atm) spanning recent concentrations to the high end predictions for 2100 (IPCC). *U. lactuca* is a fast growing species with a complex CCM that uses HCO<sub>3</sub><sup>-</sup> to saturate photosynthesis with inorganic carbon. *P. cartilagineum* depends on the diffusive uptake of CO<sub>2</sub> that potentially leaves photosynthesis carbon limited. To test for responses to OA in these species that differ in their primary carbon source and carbon acquisition mechanism we cultured each alga for 21 days at various pCO<sub>2</sub> concentrations. A pH drift experiment was then performed to determine the relative activity of the CCMs if present. In both cases, growth rate was enhanced by intermediate pCO<sub>2</sub> relative to recent and very high pCO<sub>2</sub>. The CCM of *Ulva* was similarly affected by pCO<sub>2</sub>. *P. cartilagineum* showed evidence of external carbonic anhydrase activity and declining efficiency of inorganic uptake as pCO<sub>2</sub> increased. The results indicate that CCMs are regulated in response to ocean acidification. This coordinates with findings that growth increases in *Ulva* as pCO<sub>2</sub> increases, but indicates an upper threshold of this benefit.

## 2OR.6

### LIMITS TO THE POSITIVE EFFECT OF OCEAN ACIDIFICATION ON MACROALGAL PRODUCTION

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About one third of macroalgal species lack any carbon concentrating mechanism (CCM), which prevents carbon limitation under air equilibrium in other seaweed species. It is predicted that those species lacking CCM's will benefit from ongoing ocean acidification in terms of primary productivity and growth. The absolute sizes and pattern of those benefits are not known. Here, we compare the results of a model based on composite data from the literature, with a growth experiment using *Plocamium cartilagineum*, a broadly distributed rhodophyte species lacking a carbon concentrating mechanism and hypothesized to be carbon limited under current conditions. We grew *P. cartilagineum*, at 15 and 20°C in seawater aerated with a total of 53 different pCO<sub>2</sub>s (from 344 to 1053  $\mu$ atm), in 8 multi-week trials over 12 months. We measured growth and photosynthetic rates. A linear mixed model analysis was used to partition the effect sizes of drivers of variation in the experiment. The growth rates and maximum photosynthetic rates responded nonlinearly to OA, increasing with elevated pCO<sub>2</sub> from recent atmospheric level to up 450  $\mu$ atm and decreasing at higher pCO<sub>2</sub>. Light



harvesting efficiency was unaffected by  $p\text{CO}_2$  and inversely related to temperature. We were able to compare the results of the growth experiment directly to the model based on the additive effects of temperature and  $p\text{CO}_2$  on photosynthetic rates, finding concordance of

the pattern of response. The size of the effect of  $p\text{CO}_2$  on growth rate in the experiment was greater than the effect predicted by the model. These results predict that the benefit of OA for macroalgal growth may disappear as ocean acidification continues through this century.

### 3. Algal Lipids not just for burning

#### 3KN.1

#### METABOLIC ENGINEERING OF DIATOMS FOR THE ENHANCED PRODUCTION OF HIGH VALUE LIPIDS

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It is now well established that omega-3 long chain polyunsaturated fatty acids (LC-PUFAs), especially eicosapentaenoic acid (EPA; 20:5 $\Delta$ 5,8,11,14,17) and docosahexaenoic acid (DHA; 22:6 $\Delta$ 4,7,10,13,16,19) are essential constituents of human nutrition. Although marine fish are the main dietary source of EPA and DHA, the depletion of fish stocks and pollution of the marine environment indicate an urgent need for an alternative and sustainable source of omega-3 LC-PUFAs. Marine microorganisms are the primary producers of omega-3 LC-PUFAs in the aquatic food chain and EPA- and DHA-rich microalgae have been demonstrated to be a promising alternative source to fish oils. However, commercial production of high value products like omega-3 LC-PUFAs is expensive and also presents a substantial technological challenge for upscaling. There is, however, an increasing interest in the metabolic engineering of microalgae to enhance the accumulation of these important fatty acids. *Phaeodactylum tricornerutum* is a unicellular diatom, which accumulates up to 30% EPA but only traces of DHA and which is considered a good source for the industrial production of EPA. We have engineered *P. tricornerutum* to accumulate elevated levels of DHA by overexpressing heterologous genes encoding enzyme activities of the omega-3 LC-PUFA biosynthetic pathway. Our data demonstrate the efficient channelling of DHA into neutral lipids with several novel triacylglycerol species being detected in the transgenic strains. This study provides novel evidence for the potential of using metabolic engineering to optimize omega-3 LC-PUFAs content in transgenic microalgae and also provides insights into the flux of these fatty acids in diatoms.

#### 3KN.2

#### INDUSTRIAL POTENTIAL OF MICROALGAE FOR APPLICATIONS IN FOOD, FEED AND SPECIALTY CHEMICALS

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The objective of my research is to reduce the cost of algal production and biorefinery and to develop the basis of an industrial production process. Microalgae are considered as one of the most promising feedstocks for a sustainable supply of commodities and specialties for both food and non-food products. For the industrialization of algal technology the following approach is important:- Whole chain approach: integrating the full production chain.- Multidisciplinary approach: different expertise expertises need to be integrated.- Bridge from fundamental research to applications: technologies need to be developed both on a lab- and pilot- scale and move from initial idea to the production processes that deliver competitive and innovative products to our industrial partners. Our present research program is driven by a techno-economic analysis. Our first techno-economic analysis was published in 2011. Based on literature data we calculated that the cost of biomass production was nearly 6 €/kg dry matter if performed at a 100 ha scale and we needed to reduce production costs 10 times. The research agenda for the 15 years roadmap was determined by the techno-economic analysis. The techno economic analysis has been revisited in 2014. For biomass production costs experimental results from AlgaePARC pilot facility were used. In addition, costs for biorefinery have been included. Biorefinery and production costs were combined and compared to the market values. Market values resulted from different combinations of end products from microalgae and cyanobacteria.

#### 3OR.1

#### PLASTICITY OF FATTY ACID PROFILES AND CONTENTS IN SEAWEEDS

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Seaweeds have been identified as a potential alternative source of polyunsaturated fatty acids (PUFA). The fatty acid profile of macroalgae is particularly rich in omega-3 fatty acids, e.g. EPA (20:5 n-3) and SDA (18:4 n-3). The western diet is deficient in the intake of omega-3 fatty acids and a dietary supplementation of these compounds can be a powerful measure in the prevention of chronic diseases. As part of NutraMara, Ireland's Marine Functional Foods Initiative, we assess and evaluate the potential of marine-origin bioactive compounds as components in Functional Foods and as food ingredients. One focus of the project has been to achieve a better understanding of the dynamics and variability of bioactives in seaweeds in natural and under controlled laboratory conditions. A major interest here is the plasticity of fatty acids in seaweeds. It is understood that the lipid profile of seaweeds can vary strongly according to environmental conditions in their habitat. In order to achieve algal biomass which is stable and optimised with regard to chemical composition for specific uses, a better understanding of such dynamics is needed. Here, an overview of variability of fatty acids in selected brown and red macroalgae of commercial interest (*Ascophyllum nodosum*, *Laminaria digitata*, *Fucus serratus* and *Palmaria palmata*) in Ireland will be presented. The data suggest distinct species-specific seasonal and spatial patterns. Further detailed analysis of different polar and neutral lipid classes within *F. serratus* and *P. palmata* revealed species- and habitat-specific responses to environmental changes with significant implications for commercial applications. The results may support industry in the selection of target species and optimal sampling times and locations in order to apply high-value algal biomass as a source for valuable bioactive food compounds.

### 3OR.2

#### LIPID COMPOSITION MODIFICATIONS OF THE MARINE DIATOM *PSEUDO-NITZSCHIA DELICATISSIMA* UNDER COPPER STARVATION

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The effect of Cu limitation on the lipid composition of the diatoms *Pseudo-nitzschia delicatissima* was assessed in ultra-clean conditions in Aquil medium, starved or not in copper (0 or 20 nmol.L<sup>-1</sup> of total Cu). In both conditions, detailed lipid composition (membrane and reserve lipid fatty acid (FA) and lipid class compositions; sterol composition) was also analyzed, quantitatively and qualitatively; by high performance thin layer chromatography (HPTLC) and gas chromatography (GC). To understand the significance of the lipid modifications, some cell physiological parameters were monitored using flow cytometry (growth rate, mortality, morphology, chlorophyll content, cell cycle, esterase activity and total lipid content) and using a pulse-amplitude modulation fluorometer (quantum yield (QY)). Cu starvation led to marked modifications of cell lipid composition. Total lipid content was 3-fold higher in Cu-starved cells than in replete ones. This was principally attributable to higher amounts of triglycerides, which were the main reserve lipids. Qualitative composition of membrane lipids was also profoundly modified, with an inversion of the relative proportions of cholesterol and 24-methylene cholesterol, and a 70%-increase of digalactosyl diacylglycerol/monogalactosyl diacylglycerol ratio. In the meantime, Cu starvation led to important physiological changes. Cell cycle was blocked in G2 phase, which led to a 34%-reduction of cell growth rate. Observed increased triglyceride cell content could thus reflect energy storage in absence of cell division. Cu starvation also induced a slight QY decrease (-9%), compensated by a higher chlorophyll content, which allowed maintaining cell energy acquisition. Observed re-arrangements of *P. delicatissima* membrane lipids could partly explain why Cu starvation only induced a slight QY decrease. It would allow maintaining optimal micro-environment for membrane proteins, some of them playing important roles in photosynthesis, and thus their optimal activity.

### 3OR.3

#### LINKAGE BETWEEN PHOTOSYNTHESIS AND NITROGEN METABOLISM ON THE ACCUMULATION OF LIPIDS IN MICROALGAE

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Photosynthetic microalgae have a great potential for biofuel production. In microalgae, both growth and lipid production are a physiological trade-off, resulting from balancing the resource and energy allocation within the cells amongst the anabolic and catabolic pathways. In this sense, lipid production in microalgae has been suggested to act as a sink of the chemical energy and reductant power resulting from photosynthesis when cell growth is arrested. Therefore, in order to better understand the lipid production pathway, the linkage between both photosynthesis and nitrogen assimilation was investigated in the present research. Two taxonomically different species (*Nannochloropsis oceanica* and *Phaeodactylum tricorutum*) of microalgae were grown in batch cultures at high light intensity and under different nitrogen sources (Nitrate, Nitrogen free and Ammonia). Changes in the growth rate, biochemical composition and photophysiology by Chlorophyll *a* fluorescence methodology were examined to establish trade-offs in their physiological response towards biofuel production. Both species presented a significant decrease in growth rate in the Nitrogen free treatment with respect to the Nitrate and Ammonia, matching the decrease in Chlorophyll *a* and protein contents. Conversely, total fatty acids were higher in the Nitrogen free treatment, but similar to the content obtained in the Ammonia treatment. In both species, Chlorophyll *a* fluorescent measurements indicated the photodamage of the Photosystem II in the Nitrogen free treatment (significant lower maximum quantum yields and photosynthetic efficiencies), while marked differences in the amount of photochemical (qP) and non-photochemical quenching (NPQ) were found between them. The role of the NPQ on lipid production was further investigated.

### 3OR.4

#### THE EUROPEAN PUFACHAIN PROJECT (FP7) - A VALUE CHAIN FROM ALGAL BIOMASS TO LIPID-BASED PRODUCTS

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PUFACHain aims at substantiating the industrial development of sustainable high-value products from microalgae, i.e. mainly the omega-3 polyunsaturated fatty acids DHA and EPA. Two bioresources, the SAG (Göttingen University) and the CCCryo (Fraunhofer) culture collections, will provide optimization through screening of algal strains from temperate and the Polar Regions. The cold-adapted strains may sustain a sufficient PUFA outdoor production in Middle Europe in the colder season. At SAG, those strains, which were preselected through a previously published large qualitative screening for lipid profiles and appear promising for photobioreactor technology, are further tested for their optimal PUFA yield. This includes many strains not exploited so far as well as already well-known EPA/DHA producers. So far, the EPA, DHA and ARA content in dry algal biomass was quantified for more than 100 strains, representing almost all classes of eukaryotic algae with emphasis on Chlorophyta, Eustigmatophyceae (Stramenopiles) and Dinophyta. PUFA content varied greatly within a certain group among different species. EPA ranged from 0.04% to 4.6% of dry mass and DHA from 0.03 to 1.8%. There were even cases where the PUFA content varied between different strains within the same species. Almost all strains contained ARA, which, however, is undesired due to its inflammatory activity in human organism. However, a selection of strains with high EPA and/or DHA content and no or low ARA content was recovered and will be further proceeded to next steps of PUFA yield optimization, upscaling and extraction following the biorefinery concept.

### 3OR.5

#### LIPID ACCUMULATION IN *PHAEODACTYLUM TRICORNUTUM* OBTAINED BY A MUTATION-SELECTION PROCEDURE

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Microalgae are currently emerging as one of the most promising alternative sources for the next generation of food, feed and renewable energy in the form of biofuel. Major challenges for the development of this resource are to select lipid-rich strains using high-throughput staining for neutral lipid content in microalgae species. Species domestication strategies



including random mutation and selection procedure is a powerful tool to genetically modify organisms and enhance the lipid productivity. During the Facter 4 project we aimed to develop a high-throughput strategy to obtain rich-lipid microalgae strains with a mutation-selection approach. In this study, two mutagenic chemical agents were tested: ethyl methanesulfonate (EMS) and the acridine mutagen (ICR191) that induce random alkylation and intercalation in microalgal DNA. Lethal mutagenic concentrations were first assessed with the diatom *Phaeodactylum tricorutum*. Then, a high throughput method based on factorial design was developed that allowed to test a variety of combinations for the mutagenic agent concentration and the exposure duration. Chl a fluorescence, optical densities (OD680nm and OD800nm) in the resulting populations were monitored in 24-well plates to statistically assess survival and growth rates. Results showed that exposure duration for ICR treatment did not affect survival rate contrary to EMS treatment. Survival rate was a decreasing and linear function of the ICR and EMS concentrations inside the experimental domain. Five *P. tricorutum* mutant populations for each mutagen were selected and showed survival rates from 1% to 90%. For the five populations, cells were stained with the Nile red dye and cells demonstrating the 10% highest Nile red fluorescence were then sorted and collected by flow cytometry previous to lipid analysis. The selected populations we exposed to a second round of mutation-selection and screened for their fatty acids content (total, neutral and polar lipids) and change in fatty acid classes.

### 3OR.6

#### CHARACTERIZATION OF LIPIDS IN ALGAE UTILIZING AN IMAGING FLOW CYTOMETER (FLOWCAM)

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Establishing optimal micro algae production conditions and harvesting methods is a challenge in Algae Technology Industries. The ability to effectively characterize the lipid content of algae and the effectiveness of bio-reactors is essential to tipping the balance in the cost effectiveness of algae production. Here we present a semi-automated method that takes advantage of the fluorescence detection and measuring capabilities of an imaging flow cytometer (FlowCam) to measure the relative lipid content in algal cells. Used in conjunction with the Nile Red stain, the instrument both detects and measures lipid content in cells, allowing the user to rapidly gauge lipid production in algal cultures. This presentation will outline the method of performing lipid production characterization through this imaging

system. Building upon advances in technology, input from the user community, and expanded resources, engineers at Fluid Imaging Technologies have recently designed the 4thGeneration FlowCam, informally referred to as the 'High Sensitivity FlowCam'. With more precise fluorescence data regarding intensity, size, and concentration information, this new platform brings about a higher level of insight into lipid production in algal cells.

### 3OR.7

#### UNLOCKING NATURE'S TREASURE-CHEST: SCREENING FOR OLEAGINOUS ALGAE

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Micro-algae synthesize high levels of lipids, carbohydrates and proteins photoautotrophically, thus attracting considerable interest for the biotechnological production of fuels, environmental remediation, functional foods and nutraceuticals. Currently, only a few micro-algae species are grown commercially at large-scale, primarily for "health-foods" and pigments. For a range of potential products (fuel to pharma), high lipid productivity strains are required to mitigate the economic costs of mass culture. Here we present a screen concentrating on marine micro-algal strains, which if suitable for scale-up would minimise competition with agriculture for water. Mass-Spectrophotometric analysis (MS) of nitrogen (N) and carbon (C) was subsequently validated by measurement of total fatty acids (TFA) by Gas-Chromatography (GC). This identified a rapid and accurate screening strategy based on elemental analysis. The screen identified *Nannochloropsis oceanica* CCAP 849/10 and a marine isolate of *Chlorella vulgaris* CCAP 211/21A as the best lipid producers. The most productive diatom strain was *Cylindrotheca fusiformis* CCAP 1017/2. Analysis of C, N, protein, carbohydrate and Fatty Acid (FA) composition identified a suite of strains for further biotechnological applications e.g. *Dunaliella polymorpha* CCAP 19/14, significantly the most productive for carbohydrates, and *Cyclotella cryptica* CCAP 1070/2, with utility for EPA production and N-assimilation.

### 3OR.8

#### IONIC LIQUID ASSISTED SUBCRITICAL WATER PROMOTES THE EXTRACTION OF LIPIDS FROM WET MICROALGAE SCENEDESMUS SP.

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Presently, microalgae are considered one of the most promising feedstocks. However, a large scale and efficient extraction method is costly to implement. Therefore, in this paper, we examine the use of an environmentally friendly solvent-ionic liquid assisted subcritical water method to extract lipids from wet *Scenedesmus* sp. The extraction efficiency under various experimental conditions was evaluated. The optimal experimental parameters consisted of the ionic liquid species [HNEt<sub>3</sub>][HSO<sub>4</sub>], a 1% concentration of ionic liquid species- [HNEt<sub>3</sub>][HSO<sub>4</sub>], and a temperature of 110°C. The lipid yield under the optimal conditions (35.67% dried weight biomass) was close to that of the Bligh & Dyer method (35.32% dried weight biomass). However, the triacylglycerol content (73.63% total lipids) was higher than that of the Bligh & Dyer's method (60.44% total lipids). In addition, algae cell residues assembled into microspheres, therefore, they were easy to separate from the mixture. Generally, the new extraction method is environmentally friendly, efficient and easy to scale up.

### 3OR.9

#### ALGAE ARE THE CENTERPIECE OF WORLD AQUACULTURE, FEEDING FISH AND CLEANING WATER

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'Green water' plankton - microalgae, bacteria, fungi, protozoa and small zooplankton – lead aquaculture. Over 200 million metric tons y<sup>-1</sup> (mmt y<sup>-1</sup>) of microalgae are cultured in artificial water impoundments, fertilized and carefully stoked by mixed fish species

(Neori 2013). Filter feeder fish (world production ~ 10 mmt y<sup>-1</sup>) ingest 12-30 ton plankton to produce a ton of fish. 'Green water' is the basis of polyculture – a leading form of aquaculture. It is sustainable and profitable, thanks to the dual function of the plankton in taking care of the two main processes in aquaculture – production of food and maintaining water quality. The plankton is a low cost fish feed, whose production involves environmental services of waste disposal and water oxygenation. Therefore, polycultured fish, most of them planktivorous, supply cheap seafood for large markets. In the top ten animal species aquacultured in 2012 (FAO statistics), seven were low-value greenwater carps, tilapia and clams. The cultured planktivorous bivalves (~15 mmt y<sup>-1</sup>) ingest 15-25 ton natural plankton to produce a ton. The total quantity of algae used in aquaculture, therefore, surpasses by many folds total world aquaculture production reported by the FAO. The operation of polyculture fishponds is fine-tuned according to climate, local species, resources, markets etc. Proper fish species diversity with complementary feeding habits, added to well-controlled rates of waste loading and water exchange, attain a balanced feeding of the different plankton and plant categories. The result is an optimal use of pond resources and maximal the farm's output. The supply of 'natural' plankton to coastal bivalve aquaculture is also influenced by coastal eutrophication, farm location and design. Increased scientific attention to 'green water' plankton may cost-effectively and sustainably raise world seafood production. Neori, A, 2013. 'Greenwater' aquaculture: the largest aquaculture sector in the world. *World Aquaculture* 44 (2): 26-30

### 3OR.10

#### MICROALGAE AS A RENEWABLE RAW MATERIAL CULTIVATED IN AN URBAN AREA

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The world's first algae house in Hamburg, Germany was established in 2013. The innovative and sophisticated system enables an energy concept that brings together, in one circuit, solar energy, geothermal energy, a condensing boiler, district heating, and the production of microalgal biomass in the bioreactor façade. The 129 unique photobioreactors that cover an area of 200 m<sup>2</sup> produce biomass for energy

production and also research applications. In this study, the biomass obtained in the photobioreactors was harvested and eventually converted under supercritical water conditions into gaseous energy-rich molecules (mainly  $H_2$ ,  $CH_4$  and  $C_2H_6$ ). Gasification efficiencies of the biomass up to 98% were reached. With regard to the scarcity of nutrients and their global depletion within the next generations, it is necessary to find solutions that allow the regeneration of these nutrients that are used for agricultural biomass production. Therefore, the effluent water of the hydrothermal conversion was divided into two fractions

containing the nutrients of the microalgal biomass. The green algae *Acutodesmus obliquus* was cultivated with this nutrient source in order to assess the biomass parameters and fatty acid productivity. Biomass and fatty acid productivity of algae grown on the effluent water reached up to  $1.3 \text{ g L}^{-1}\text{d}^{-1}$  and  $312.9 \text{ mg L}^{-1}\text{d}^{-1}$ , respectively, and were comparable to control medium. These results indicate that the residual water from hydrothermal gasification can be partially applied as a sole nutrient source for large scale production of algal biomass and be a sustainable substitute for commercial fertilizers.

## 4. Algae-microbiome interactions: integrative overview from biology to chemistry

### 4KN.1

#### ARE BIOLOGICAL CONTROLS OF HARMFUL ALGAL BLOOMS STABLE OVER TIME?

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Several active microeukaryotic parasitoids are known to be efficient biological controls of harmful algal blooms. These parasitoids, which belong to different eukaryotic lineages, have a convergent live cycle strategy. Infection processes generally end in 2-4 days by the death of the unicellular host and the production of hundreds of free-living zoospores released into water, ready to initiate a novel infection. This mode of propagation well explains how such parasitoids can control their host populations during blooms. These parasitoids are specialist to generalist, but generally failed to infect all host strains cross-infect in culture. Complex coevolutionary processes have been observed between the host and its parasitoids even during the short period lasts by a bloom (few weeks). Inter-annual monitoring survey determined that these biological controls are rather unstable over time. The capacity of harmful algal species to escape parasitoids and blooms results from fragile equilibriums that could be frequently upset by human activities.

### 4KN.2

#### MICROBIOMES IMPACT ALGAL ACCLIMATION: THE EXAMPLE OF A FRESHWATER STRAIN OF *ECTOCARPUS*

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Increasing evidence exists that bacterial communities interact with and shape the biology of algae, and that their evolutionary histories are connected. Considering interactions between algae and associated bacteria is therefore a key to understanding the biology and evolution of these organisms. A striking example for this is the brown algal genus *Ectocarpus*, which comprises a strain that has recently colonized freshwater, a rare transition in this lineage. Our results demonstrate that this freshwater strain depends on bacteria for this transition: cultures that have been deprived of their associated microbiome do not survive the transfer to freshwater, but restoring the associated microflora also restores the capacity to acclimate to this change. We generated an inventory of bacteria associated with this alga when cultivated in different salinities, and show that the bacterial community composition was affected by this abiotic factor. Examining a range of other closely related algal strains, we observed that the presence of two bacterial operational taxonomic units correlated significantly with an increase in low salinity tolerance of the alga. Despite differences in the community composition, no indications were found for functional differences in the bacterial metagenomes predicted to be associated with algae in the different salinities tested, suggesting functional redundancy in the associated bacterial community. To study complex 'holobiont' system such as that of the freshwater strain and its associated microbiome in changing environmental conditions, we propose a metabolism-centered approach that uses combined metabolic models of both algae and bacteria. First examples of how these approaches may be implemented in *Ectocarpus* will be given in the talk. We anticipate this approach will prove valuable to study algal-bacterial interactions both in controlled laboratory- and, in the long run, in natural conditions.



**4OR.1****THE MICROBIOME OF A SEAWEED INVADER ACROSS ITS INTRODUCED EUROPEAN RANGE**

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Anthropogenic activities result in environmental and ecological changes from local to global scales, with biological invasions, climate change and habitat destruction among the most problematic ones. Through various pathways, these activities alter marine ecosystems from the smallest to the largest scale. Research on how organisms cope with stress and environmental change has traditionally focused on individuals and populations. Genetic diversity is important for the success of populations in adapting to changing environmental factors. Introduced species are faced with tremendous challenges when introduced beyond their native range. Not only do they have to cope with new environmental conditions, competitors and predators, mostly their populations are small and with very limited genetic diversity. The holobiont and hologenome theories pose that the diversity of associated microbes and viruses provide a range of services to their host that are important for performance and adaptation. In this study, we explore bacterial communities associated to one of the most successful invasive seaweeds of our time using next generation amplicon sequencing of the 16S rRNA gene. We hypothesize that associated bacterial communities might play a role on the success of their host, as bacteria can affect the morphogenesis, development, growth, metabolism and the immune system of their algal host. Our descriptive approach exploring the diversity and composition of *Sargassum muticum* associated bacterial communities across European latitudinal gradient covering both northern (Norway) and southern (Morocco) invasion fronts aims to show to what extent microbiomes change geographically or with invasion phase and among different tissue types of the host. We apply a metagenomic approach to provide functional information, and experimental manipulations of CO<sub>2</sub> and grazers to reveal adaptative aspects of the associated microbiomes.

**4OR.2****AN IMPLICATION OF BACTERIA-DIATOM INTERACTION AT THE ONSET OF SEXUAL REPRODUCTION IN DIATOMS**

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Sexual reproduction of an araphid pennate diatom *Pseudostaurosira trainorii* has been observed in detail and the involvement of sex-pheromone at its onset was experimentally documented as follows: (1) vegetative cells of female clone release their sex pheromone ph-1 when they are in the sexual size window; (2) when a male cell detects the pheromone ph-1, the cell is sexualized to produce and release the gametes; (3) at the same time, the male pheromone ph-2 is also secreted to let the female sexualized and releases the gamete (egg). Although this reciprocal pheromone interaction must take place at the onset of sexual reproduction at natural conditions, I recently found the fact that it is also possible to artificially stimulate sexualization of male cells solely by antibiotics treatment with the absence of female clone or its sex pheromone. Bacterial involvement in this phenomenon is suggested since the gamete formation is not reproducible with axenic culture. Presence or absence of the induction of gamete formation with antibiotics is mainly depending on the classes of antibiotics added to the culture medium. Most of aminoglycosides, which inhibit protein synthesis, are “positive” (i.e. treatment stimulates the release of gametes), whereas the other classes are generally negative, with a few exceptions such as zeocin (glycopeptides) and tetracycline (tetracyclines) that display positive results, albeit weakly. Among aminoglycosides, streptomycin is the most effective, followed by gentamicin, hygromycin B and neomycin. The other aminoglycosides, such as kanamycin, geneticin (G418), nourseothricin, are also shown to be moderately positive. A comparative metabarcoding analysis of bacterial flora with/without antibiotic treatment is currently ongoing and the result will be presented.

**4OR.3****PHYLOGENETIC DIVERSITY OF EPIBIOTIC GRAM-POSITIVE BACTERIA ISOLATED FROM ANTARCTIC MACROALGAE**

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The Antarctic continent is of exceptional biological interest, possessing a rich diversity of marine macroalgae and a high endemism. However, very little is known about the diversity and bioactive potential of bacteria associated with the surface of Antarctic macroalgae. Antarctic macroalgae are an untapped source of microbial diversity and represent a promising source of novel therapeutic agents. This study aims to explore the phylogenetic diversity and antimicrobial activity of Gram-positive bacteria isolated from the surface of intertidal and subtidal Antarctic macroalgae. Gram-positive bacteria are well-known producers of secondary metabolites. Representative species of green (*Monostroma hariotii*), red (*Iridaea cordata*, *Phycodrys antarctica*, *Pyropia endiviifolia*) and brown algae (*Adenocystis utricularis*) were collected from the intertidal and subtidal zone (5 and 30 m) of King George Island, Antarctica, in January 2014. Surface-associated bacteria were investigated by cultivation-based methods and 16S rRNA gene sequencing. Epibiotic bacteria were screened for antibacterial activity against a panel of 6 antarctic macroalgae-associated microorganisms. In total, 50 Gram-positive strains were isolated, which were affiliated to 12 families of bacteria, predominantly Actinobacteria such as Micrococcaceae, Microbacteriaceae, Nocardiaceae, Dermabacteraceae, Pseudonocardiaceae and Streptomycetaceae. Antimicrobial activity was found in isolates of *Agrococcus*, *Brachybacterium*, *Citricoccus*, *Kocuria* and *Streptomyces*. This study shows a high diversity of Gram-positive bacteria on Antarctic macroalgae as well as highlights their potential to produce antimicrobial compounds. Acknowledgements: This study was supported by Grant RT\_06-13 from the Instituto Antártico Chileno (INACH). We thank the INACH staff at Station Prof. Julio Escudero for logistic support. Special thanks to Dr Iván Gómez and his group (Proyecto Anillo ART1101) for its valuable support during the field work.

#### 4OR.4

##### AUSTRALIAN NATIONAL ALGAE CULTURE COLLECTION – UNIQUE BIODIVERSITY INFORMING ECOSYSTEM KNOWLEDGE AND BIORESOURCES

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The Australian National Algae Culture Collection, ANACC, maintains over 1000 strains of microalgae from Australian marine, estuarine and fresh waters from the tropics to Antarctica. As one of the six National Research Collections Australia, it is a significant repository of characterised Australian algal biodiversity. ANACC has developed along with CSIRO's research programs addressing national priorities, with strains having relevance to environmental issues and the development of microalgae as a bioresource. Interfacing ecosystem knowledge with bioresource potential is a focus for ANACC. Studying the interactions of environmentally significant bloom forming microalgae with bacteria has demonstrated the importance of considering algal growth dynamics within the microbiome. We have demonstrated highly synergistic growth dynamics of a *Marinobacter* strain with the toxic dinoflagellate *Gymnodinium catenatum* whereas no such synergy was seen with another bacterial strain, also isolated from cultured *G. catenatum*. It was also found that the bacterial community can alter intracellular paralytic shellfish toxin production of dinoflagellates, most likely to be due to bacterial effects on dinoflagellate physiology rather than bacterial biotransformation of toxins. Another ecologically significant microalga is the green alga *Botryococcus braunii* that forms colonies held together by a lipid biofilm matrix. This species produces high amounts of hydrocarbons and is of great interest as a source of biofuels. However, its slow growth hampers such bioresource development. We have isolated and characterized *Botryococcus* strains from populations spanning tropical to temperate Australia. Chemical Races A, B and L have been identified including one population consisting of two Races. Investigations into growth of *Botryococcus* include the bacterial communities associated with the cultured strains. Our results demonstrate the importance of considering the interactions of microalgae within the microbiome as a key part of ecosystem knowledge and bioresource development.

#### 4OR.5

##### DIFFERENTIATION IN BACTERIAL COMMUNITIES ASSOCIATED TO THE RED SEAWEED GENUS *ASPARAGOPSIS* IN THE WESTERN ATLANTIC AND THEIR POTENTIAL DRIVERS

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Biological invasions can induce harmful alterations, from genes to ecosystems, and are considered one of the most harmful components of global change. The genetic pool of introduced species greatly influences their capacity to establish and expand. The hologenome theory states that associated microorganisms supply their host with extra capabilities that might lead to faster adaptation and positive response to the new invaded environment. A relative low but increasing number of studies have provided evidence that bacteria associated to macro-organisms determine for a large part the well-being of their host, making them a potentially essential element of evolution and adaptability of the holobiont. Among the 100 worst invasive alien species and introduced in many places around the globe are representatives of the red seaweed genus *Asparagopsis*, which includes two different species, *A. armata* and *A. taxiformis*. Both have a haplo-diplontic, heteromorphic life cycle with alternating haploid gametophytes and diploid sporophytes. *A. armata* (a genetically homogeneous taxon) is a temperate species native to southern Australia and New Zealand, whereas *A. taxiformis* is a cryptic species complex composed of at least five genetically distinct lineages, each characterized by a distinct geographical distribution. Here, we compare bacterial communities of both *Asparagopsis* species including the haplodiplont stages of *A. armata* and two lineages of *A. taxiformis* in the West Atlantic from mainland Portugal to oceanic islands. In addition, we investigate the invasive potential, based on associated bacteria putative function, of the two invasive taxonomic entities in South Portugal. Preliminary results show that the cryptic separation of *A. taxiformis* lineages is also supported by their bacterial community composition and, invasive taxa in South Portugal, display a wider selection of bacterial OTUs that might be involved in the adaptation of the invasive taxa.

#### 4OR.6

### LIGHT AND DISSOLVED ORGANIC CARBON DRIVE INTERACTIONS WITHIN RIVER MICROPHYTOBENTHIC COMMUNITIES

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The dominant primary producers in headwater rivers are microphytobenthic communities. As well as playing an important role in primary productivity, these communities of algae, bacteria, fungi, and other microorganisms also play a larger role in nutrient transformation at the sediment-water interface. These communities are however subject to interactions which may influence this important ecosystem service. One such interaction – trophic coupling – provides a positive feedback loop allowing high biomass to occur in low nutrient/DOC conditions. However, this is disrupted in contrasting environments where DOC is not limiting. We aim to examine these microphytobenthic communities by characterising them across a gradient of river sub-catchment geology types. Here we present results from a study of soft sediment microphytobenthic communities in the Hampshire Avon river catchment, United Kingdom. Multiple rivers from different clay, greensand and chalk geology sub-catchments were sampled in a spatial-temporal design throughout 2013. Results showed that these rivers represent a natural light/DOC gradient, which influenced the overall trophic dynamics of the microphytobenthic communities. Algal biomass was higher in chalk sub-catchments, while diatom community composition showed distinct differences in species richness, diversity and species present between net-autotrophic and net-heterotrophic systems. These patterns were replicated in algal EOC production; multiple carbohydrate fractions are positively correlated to DOC concentrations, however they showed no relationship to other macronutrients such as nitrate & phosphate. Net autotrophic communities showed significant flux of nitrite from the sediment to the water column, and a similar flux of phosphate – both of which were not seen in the heterotrophic communities. Positive correlations were observed between river DOC and sediment EOC, which suggests that trophic coupling state depends on DOC concentration. High DOC/EOC ratios and light controlled incubation experiments infer that in high DOC rivers, bacteria are able to outcompete algae for macronutrients, leading to microphytobenthic communities shifting from net-autotrophy to heterotrophy.

#### 4OR.7

### EFFECTS OF FUNGAL PARASITISM ON FRESHWATER CYANOBACTERIAL BLOOMS

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Many species of phytoplankton are susceptible to parasitism by fungi from the phylum Chytridiomycota (i.e. chytrids). However, few studies have reported the effects of fungal parasites on filamentous cyanobacterial blooms. To investigate the missing components of bloom ecosystems, we examined an entire field bloom of the cyanobacterium *Anabaena macrospora* for evidence of chytrid infection in a productive freshwater lake, using a high resolution sampling strategy. *A. macrospora* was infected by two species of the genus *Rhizosiphon* which have similar life cycles but differed in their infective regimes depending on the cellular niches offered by their host. *R. crassum* infected both vegetative cells and akinetes while *R. akinetum* infected only akinetes. A tentative reconstruction of the developmental stages suggested that the life cycle of *R. crassum* was completed in about 3 days. The infection affected 6% of total cells (and 4% of akinetes), spread over a maximum of 17% of the filaments of cyanobacteria, in which 60% of the cells could be parasitized. Furthermore, chytrids may reduce the length of filaments of *Anabaena macrospora* significantly by “mechanistic fragmentation” following infection. All these results suggest that chytrid parasitism is one of the driving factors involved in the decline of cyanobacteria blooms, by direct mortality of parasitized cells and indirectly by the mechanistic fragmentation, which could weaken the resistance of *A. macrospora* to grazing.

#### 4OR.8

##### PHAEOVIRUSES EXTEND THEIR HOST RANGE TO THE KELPS

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Phaeoviruses infect the brown algae, Phaeophyceae. They exploit a Persistent evolutionary strategy via genome integration and are the only known representatives to do so amongst the giant viruses, *Megaviridae*. Here I will present data showing an increase in phaeovirus prevalence among brown algae, which has led to alternative strategies of infection. I will also show how exceptional phaeoviruses

are compared to most host-virus systems. Given what we now know about phaeoviruses, unlocking the hidden diversity within other seaweed systems has the potential to fundamentally change concepts within Virology and Phycology alike.

#### 4OR.9

##### LIFE CYCLE STRATEGIES OF A LARGE VIRUS THAT INFECTS THE BLOOM FORMING *EMILIANIA HUXLEYI*

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The coccolithophore *Emiliania huxleyi* forms massive blooms in the oceans that are terminated by a large double-stranded DNA coccolithovirus, the *Emiliania huxleyi* virus (EhV). The cellular mechanisms that govern the replication cycle of this virus are largely unknown. Here we show that hallmarks of autophagy, a lysosomal degradative pathway that degrades undesirable or damaged macromolecules, are highly induced during the lytic phase of *E. huxleyi* viral infection. In addition to upregulation of a suite of autophagy related genes, we observed a host encoded autophagy related protein in EhV virions, demonstrating the pivotal role of the autophagy-like process in viral assembly and egress from the cells. We further investigated the modes of viral entry and egress from the cells and explored the possibility of multiple phenotypes associated with the life cycle of EhV. We measured membrane lipid composition, virion ultrastructure, and infectivity and decay rates, all pointing to the different strategies of viral infection. We propose that the pleomorphic nature of EhV will have major implications to their ecological role during host-virus dynamics within oceanic algal blooms.

#### 4OR.10

##### DISEASE RESISTANCE IN BROWN ALGAE: MECHANISMS AND HERITABILITY

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Brown algae (Phaeophyta), in addition to having an important ecological role as predominant primary producers in temperate and cold coastal seas, are of increasing relevance to the rapidly-expanding aquaculture industry. Like any other organism, brown algae are plagued by a variety of phylogenetically different pathogens. However, the mechan-

isms of disease resistance in brown algae are poorly known, in contrast to the well-studied hypersensitive response in terrestrial plants. We previously established a suitable experimental pathosystem and identified clonal brown algal strains that exhibit differential disease susceptibility to the obligate oomycete pathogen *Eu. dicksonii*. Here we show that resistance to infection is evoked by the hypersensitive death of single algal cells attacked by *Eu. dicksonii*. These cells express the programmed cell death marker metacaspase. Further conserved markers of the hypersensitive response include the deposition of blue-fluorescent metabolites and beta-1, 3-glucan, the production of reactive oxygen species, and the fragmentation of DNA characteristic for programmed cell death. This hypersensitive response was observed in ten algal species belonging to four different orders, demonstrating its broad conservation among brown algae (Phaeophyta). In addition to the description of disease resistance mechanisms we have furthermore investigated the heritability of this trait on our model pathosystem using several genealogies of *Ectocarpus fasciculatus*.

## 5. Phylogenomics: new approaches to solving old problems in algal evolution

### 5KN.1

#### UNTANGLING THE WEB OF EUKARYOTIC PHOTOSYNTHETIC EVOLUTION

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Efforts to arrive at a clear understanding of the history of eukaryotic photosynthesis have been hindered by two overarching problems. First, phylogenomic analyses have not resolved how a number of organisms containing plastids are related to one another, or to other organisms that are not photosynthetic. Such investigations are complicated by the global impacts of various uncharacterized sources of bias, which can directionally mislead tree-building algorithms and result in phylogenetic artifacts. Perhaps more challenging, however, is the second major problem, the massive horizontal movement of genes among divergent eukaryotic lineages through the process of endosymbiotic gene transfer (EGT), which involves correlated inheritance that is incongruent with the evolutionary history of the host organisms' nuclear genomes. Rigorous detection of EGT can be difficult using phylogenetic methods, particularly since widely accepted views of organismal relationships have been estimated without a clear understanding of the amount and impact of EGT on the genomes under investigation. Consequently, hypotheses of plastid evolution inferred from conflicts among gene trees can be tautological and, therefore, cannot be falsified using standard methodologies. This presentation will explore alternative approaches in comparative genomics that could more clearly articulate the tangled path of plastids through the eukaryotic tree-of-life. It also will address both philosophical and methodological considerations that can promote new breakthroughs and a better appreciation of the complex evolution of eukaryotic photosynthesis.

### 5KN.2

#### INTERRUPTED RESPIRATORY CHAIN IN THE MITOCHONDRION OF *CHROMERA VELIA*

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Apicomplexan parasites are known to possess highly reduced mitochondrial genomes encoding only three protein genes (*cox1*, *cox3*, *cob*) and fragmented rRNA genes. We investigated the mitochondrion of alveolate algae *Chromera velia* and *Vitrella brassicaformis*, the closest known phototrophic relatives to Apicomplexa. Genome, transcriptome and enriched mtDNA fractions were sequenced using Illumina platform. It appears that while *Vitrella* displays the same three mitochondrial protein genes as apicomplexan mitochondrion, *Chromera* mitochondrion holds a genome with the smallest known coding capacity containing just two protein genes (*cox1*, *cox3*) and fragmented rRNA genes. Consequently, the respiratory chain is reduced by the absence of complexes I (NADH:ubiquinone oxidoreductase) and III (ubiquinol: cytochrome c oxidoreductase). The respiratory chain is broken into two parts functioning independently. Electrons from the first part (complex II, alternative NADH dehydrogenases, dihydroorotase, electron-transfer flavoprotein etc.) are channeled to ubiquinone, which passes them to alternative oxidase, an electron sink, without any consequent proton pumping and link to the respiration whatsoever. Electrons for complex IV are provided by unique combination of L and D lactate cytochrome c oxidoreductases. In contrast, *Vitrella* still has the complex III but alternative enzymes are already available in the genome. It appears that most of non-canonical enzymes involved in the respiratory chain, which were found in chromerid algae, are of early eukaryotic origins.

### 5KN.3

#### EVOLUTION OF PLASTID-TARGETED PROTEINS IN THE SECONDARY PLASTID-CONTAINING MIXOTROPHIC EUGLENID - *RAPAZA VIRIDIS*

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Plastids of autotrophic euglenids originated through a secondary endosymbiosis. A heterotrophic euglenid engulfed a prasinophyte-like green alga, most likely *Pyramimonas*, and maintained it as a permanent endosymbiont. This finding is supported by numerous molecular phylogenies. However, molecular data from green algae and euglenids are limited and phylogenetic relationships are mainly inferred from plastid-encoded genes, thus exact green algal donor and early evolution of euglenid plastids are still uncertain. *Rapaza viridis*, a recently described mixotrophic euglenid, might shed new light on euglenid endosymbiosis. *R. viridis* possesses functional chloroplasts and consumes a specific strain of prasinophyte alga, *Tetraselmis* sp. Phylogenetic analyses and ultrastructural data demonstrated the intermediate features between phototrophic euglenids and phagotrophic lineages. The basal position within autotrophic euglenids suggested that *R. viridis* is the best candidate to illuminate original traits of euglenid plastids. We analysed transcriptome obtained from *R. viridis*. From 107,092 assembled sequences, 8,875 are complete at the 5' terminus, based on the presence of specific euglenid splice-leader. Out of these, 72 transcripts encoding putative plastid-targeted proteins were identified based on homology to plastid proteomes and on their involvement in processes known to be localized in the plastid. Majority of them, as we expected, shows homology to green-algal-plastid proteins and is probably derived from the plastid donor. We also predicted plastid-targeting leader sequences of over 200 transcripts using TargetP, however majority of the identified plastid-targeted proteins did not have a predicted plastid-targeting leader sequence. For better predictions of targeting to secondary plastids of *R. viridis*, a more specific method has to be developed. Further phylogenetic analyses containing data from other euglenids and green algae will decipher evolutionary history of plastid-targeted proteins and euglenid plastid as such.

## 5OR.1

### A COMPARATIVE ANALYSIS OF MITOCHONDRIAL GENOMES IN EUSTIGMATOPHYTE ALGAE

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Eustigmatophyceae (Ochrophyta, Stramenopiles) are a small algal group with species of the genus *Nannochloropsis*, being their best studied representatives due to their potential in biotechnological applications. Nuclear and organellar genomes have been recently sequenced for several *Nannochloropsis* spp., but phylogenetically wider genomic studies are missing for eustigmatophytes. Here we present a comparative analysis of mitochondrial genomes of eustigmatophytes based on previously published genome sequences from *Nannochloropsis* spp. and newly sequenced genomes from *Monodopsis* sp. (a close relative of *Nannochloropsis*), *Vischeria* sp. (a more distantly related member of the order Eustigmatales), and *Trachydiscus minutus* (representing the recently described deeply diverged clade Goniochloridales). Our analysis has yielded a number of interesting findings, five of which are especially noticeable. First, mitochondrial genomes of most eustigmatophytes are highly colinear, except that of *Vischeria* sp. that has been highly rearranged. Interestingly, this correlates with a markedly elevated substitution rate of mitochondrial genes in the *Vischeria* lineage. Second, eustigmatophyte mitochondrial genomes uniquely share a truncated *nad11* gene encoding only the C-terminal part of the Nad11 protein, while the N-terminal part is encoded by a separate gene in the nuclear genome. Third, a previously unnoticed *tatA* gene and three mitochondrial ORFs lacking discernible homologs elsewhere are conserved across the phylogenetic breadth of the class (*tatA* and two of the three unidentified ORFs are secondarily missing from *Vischeria* sp.). Fourth, *Nannochloropsis* spp. and *T. minutus* do not employ UGA as a termination codon and lack the corresponding nuclear genome-encoded release factor mRF2, indicating at least two independent simplifications of mitochondrial translation termination in eustigmatophytes. Finally, the *rps3* gene in the mitochondrial genome of *Vischeria* sp. is interrupted by the UAG codon, but the genome includes a gene for an unusual tRNA that we speculate may serve as a suppressor tRNA to properly decode the *rps3* gene.

## 5OR.2

### WHOLE GENOME PHYLOGENOMIC ANALYSIS PROVIDES DEEPER INSIGHT INTO THE ORIGINS AND DIVERGENCE OF THE CYANOBACTERIA

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Phylogenetic trees of cyanobacteria based on 16S rRNA and multilocus sequence analysis (MLSA) consistently reveal that although the cyanobacterial clade is monophyletic, sub-sections I, II and III are polyphyletic whilst terminally differentiated sub-section IV/V are monophyletic. However, these approaches do not engage with the majority of the genome that encodes more rapidly evolving or horizontally acquired genes and regulatory pathways (DNA-based and RNA-based) that may play a critical role in the biology of the cell and that are often specific to that organism/ecological niche. This is particularly important for cyanobacteria that as a group encode a surprisingly high number of proteins that have no detectable similarity to protein sequences out-with the group. Because these are not universal, this important class is not included in cyanobacterial MLSA. To address these issues and attempt to derive a cyanobacterial phylogeny that takes into account the entire cellular genome, we have applied a phylogenomic algorithm PHYLOGENOME running on a high performance compute system to perform reciprocal whole genome DNA sequence comparisons to compute genomic distances that are used to reconstruct phylogeny and perform tree reliability assessment using bootstrapping. In this study, we assembled the largest available assembly of 177 cyanobacterial completed and draft genomes distributed across 51 genera with representatives from each of morphological sub-sections I-V and generated the first comprehensive phylogenomic reconstruction of this phylum. This phylogenomic model is robust with respect to genome assembly status, base sequence errors and annotation errors. Our data indicate that marine picocyanobacteria diverged very early from the rest of the cyanobacterial groups in this study. Furthermore, our analysis shows structurally diverse sub-section II, III, IV and V cyanobacteria are likely to have arisen from unicellular ancestral cells. Sub-section IV/V (heterocystous) cyanobacteria are monophyletic and descendent from filamentous *Trichodesmium* ancestors.

### 5OR.3

#### EVOLUTION OF ALTERNATIVE PATHWAYS FOR VITAMIN C SYNTHESIS FOLLOWING PLASTID ACQUISITION

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Ascorbic acid (vitamin C) plays an important role in protecting photosynthetic eukaryotes against reactive oxygen species derived from the chloroplast. However, the role of ascorbate in plastid acquisition is unclear, as the evolutionary origins of ascorbate biosynthesis remain unknown. Most eukaryotes require ascorbate, but multiple animal lineages have become ascorbate auxotrophs due to the loss of the terminal enzyme in the biosynthetic pathway, L-gulonolactone oxidase (GULO). GULO produces hydrogen peroxide, which may have provided selective pressure for its repeated loss. GULO is absent from land plants and *Euglena*, which use an alternative terminal enzyme, L-galactonolactone dehydrogenase (GLDH) in their respective pathways. The evolutionary processes resulting in the presence of these multiple pathways are not well understood. We present evidence that GULO has been lost and replaced by GLDH in almost all photosynthetic eukaryotes. We find that *GULO* and *GLDH* have a mutually exclusive distribution and surprisingly identify *GULO* in basally derived members of the Archaeplastida (Plantae). Red algae use a modified form of the pathway found in plants and green algae, in which D-mannose and L-galactose are precursors. However, algae that have acquired plastids via secondary endosymbiosis demonstrate a hybrid pathway analogous to that found in animals, but using GLDH rather than GULO as the terminal enzyme. We propose that the development of the photoprotective role of ascorbate in ancestral Archaeplastida, to counter the production of reactive oxygen from the chloroplast, may have provided selective pressure to functionally replace GULO with GLDH, in order to uncouple ascorbate synthesis from H<sub>2</sub>O<sub>2</sub> production and provide a high capacity ascorbate biosynthesis pathway. The functional replacement of GULO with GLDH may therefore have played an important role in protecting the host cell against photo-oxidative stress following plastid acquisition.

### 5OR.4

#### GRACILARIOPSIS ANDERSONII GENOME ENCODES SEVERAL CLUES TO MAINTAINING A PERMISSIVE PARASITE ENVIRONMENT

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By far, the largest numbers of formerly photosynthetic parasites are found among the red algae. There are examples of extant parasitic taxa from nearly half the recognized floridiophyte orders and there appear to be nearly a hundred independent parasite lineages within these groups. Because there are so many independent evolutionary events of parasitism within the red algae and it appears that parasites have most often evolved from a shared ancestor with their hosts, this is an intriguing group to explore the evolutionary changes concomitant with a transition from a free-living to parasitic lifestyle. For much of their life history many red algal parasites live out their lives as a nucleus and a collection of mitochondria freely intermingling with the host's organelles (including the modified plastid, i.e., proplastid) within host cells. In order to live in this manner, they must be capable of altering host cell walls, establishing intracellular zones of influence, directing cytochemistry globally within the heterokaryon cells and directing nutrients into tissues where parasite nuclei exist. As the first step in understanding how the parasite relationship has repeatedly evolved in the red algae and why parasites are so well tolerated as intracellular guests, we have sequenced the genome of *Gracilariopsis andersonii* and *Gracilariophila oryzoides*, its obligate parasite with a combination of Illumina and PacBio sequencing. We have now finished assembly of the 108.4 Mbp *Gr. andersonii* genome. Among 9,363 predicted protein set for *Gr. andersonii*, we have identified the plastid-targeted and membrane-bound secretome gene sets. These genes provide promising targets for examining how gene synteny and protein similarity may facilitate initial infection and sustained compatibility between the host and parasite.

## 5OR.5

### GENOME-WIDE PREDICTION AND COMPARATIVE ANALYSIS OF TRANSCRIPTION FACTORS IN MICROALGAE

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Studying transcription factors (TFs), one of the key players in gene expression is of outstanding interest to investigate the evolutionary history of organisms through lineage-specific features (Lang *et al.*, 2010; Rayko *et al.*, 2010; Sharma *et al.*, 2013). In this kind of study, some gaps exist in term of available genome from certain lineages such as rhodophytes and haptophytes. In this study, we realized the genome-wide identification and comparison of TFs in algal lineages adding a new haptophyte genome (*Tisochrysis lutea*) and the rhodophyte *P. purpureum*. To reach this goal, a new optimized and automated pipeline for the TFs identification was developed. This pipeline combines research of similarities from known TFs (BLAST) and functional protein domains annotation using a large database containing plant, fungi, mammalian and cyanobacteria TFs (InterProScan5 and HMMER3 freewares). Using this tool, the TF complements of seven algae were established and compared to each other. This comparative study includes algae from two closely related lineages (Andersen, 2004; Moustafa *et al.*, 2009), the haptophytes (*Tisochrysis lutea*, *Emiliania huxleyi* and *Pavlova* sp.) and the stramenopiles (the Eustigmatophyceae, *Nannochloropsis gaditana* and the diatom *Phaeodactylum tricorutum*), as well as the green alga *Chlamydomonas reinhardtii* and the mesophilic red alga *Porphyridium purpureum*. The specificity of our pipeline allowed us to identify the presence of cyanobacterial TFs in the nuclear genome of algae for the first time. Moreover, our study confirms and highlights some lineage-specific features in haptophytes and stramenopiles. This work provides a basis to better understand gene regulation in *T. lutea*, a species of ecological interest because haptophytes are genetically diverse and often dominant in the planktonic photic realm.

## 5OR.6

### WHO WAS THE LAST APLASTIDIC CRYPTOPHYTE?

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Cryptophytes are a cosmopolitan group of algae. They are abundant in spring and summer, and are important food source for micro- and mesozooplankton. Around 200 of species have been described, but phylogeny and taxonomy of cryptophytes still remains uncertain. Moreover, environmental surveys revealed existence of a deep-branching clade within Cryptophyceae:

Basal Cryptophytes. They form a sister clade to photosynthetic cryptomonads. These two clades together (cryptomonads and basal cryptophytes) are a sister clade to the *Goniomonas* clade. *Goniomonas* is aplastidic, has never acquired a plastid, and is currently considered as an ancestor to plastidic cryptomonadales. Upon the discovery of basal cryptophytes, the question arose whether they constitute the most divergent plastid-containing cryptomonad clade, or a new heterotrophic group at the base of the cryptomonad tree. Here, we combined phylogenetic and environmental study to address this question. The maximum likelihood phylogeny of 18S rRNA nuclear genes confirmed placement of basal cryptophytes at the base of the cryptomonad tree. We designed a specific oligonucleotide probe, to detect members of this clade in environmental samples by Catalysed Reported Deposition-Fluorescence *in situ* Hybridization (CARD-FISH), and to observe their basic morphology. None of the cells hybridized with the new probe showed chlorophyll-a autofluorescence at 590 nm, meaning they were aplastidic. The phylogenetic analysis of 18S rRNA nucleomorph genes did not produce a sister clade to photosynthetic cryptomonads, suggesting that Basal Cryptophyceae had not acquired a plastid at all. Altogether, these results indicated that the last aplastidic ancestor of cryptomonads could have been a basal cryptophyte, and that the engulfment of a red algal symbiont might have been a more recent event than when assuming radiation of the plastidic cryptomonads directly from *Goniomonas*. If confirmed upon culturing and studying a species from basal cryptophytes, it will change the view of evolution of plastidic cryptophytes and nucleomorphs.

## 5OR.7

### WHOLE GENOME SEQUENCING OF THE ANTARCTIC GREEN ALGA *PRASIOLA CRISPA*

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The trebouxiophycean genus *Prasiola* is an excellent model for the study of ecological transitions, as it is one of the rare algal genera that includes marine, freshwater and terrestrial representatives. The generitype *Prasiola crispa*, an extremophile widely distributed in cold temperate and polar regions of both hemispheres, has strong potential to serve as a good model of the evolution of both green algae and the land plants. We are using next-generation sequencing data (Illumina technology) to obtain its complete genome sequence. Additionally, we are testing the Oxford Nanopore's MinION technology in order to complete the assembly with ultra-long reads. We are presenting the first results of our project: complete sequences of *P. crispa* organelles, the mitochondrion (100,036 nt) and the plastid genome (211,767 nt). Both have relatively low GC content (29.3% and 28.8%, respectively), as is usual among green algae. The gene content corresponds well with other sequenced trebouxiophyte organellar genomes, with the only peculiarity being the presence of the *rpl10* gene in the mitochondrion. In the plastid DNA, we discovered highly reduced remnants of the ancestral plastid inverted repeats (IRs, 851 bp) that bear no genes. There are clusters of non-algal mobile elements and unidentified open reading frames in both genomes; these elements appear to be organelle-specific.

## 5OR.8

### TACKLING RAPID RADIATIONS WITH CHLOROPLAST PHYLOGENOMICS IN THE RHODOMELACEAE

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The family Rhodomelaceae, with about 1000 species, is the most species-rich family of the red algae. Its

great diversity is probably associated with events of rapid radiation, leading to poorly resolved phylogenies when using one or a few molecular markers. In fact, a comprehensive phylogeny of the entire family has never been attempted. With the aim of constructing a robust phylogeny for this family, we have used Illumina HiSeq technology to obtain chloroplast genomes from species representing all major clades of the Rhodomelaceae. The phylogenetic tree is used to infer the evolution of functional morphological traits and the nature of the radiations. The chloroplast genomes of the Rhodomelaceae are 160-170 kb, small in size compared with other red algae, showing a reduction trend in the Ceramiales. The genomes are very compact with ca. 200 genes packed closely together. At the time of writing, the 20 available chloroplast genomes resolve the early radiation of the family, and we expect to have analyzed >40 complete genomes by the time of the conference to provide a more complete picture of the early radiation of the group. Our study demonstrates the power of organelle phylogenomics to unraveling rapid radiations. However, due to the large number of species in radiations like the Rhodomelaceae, further work will be needed to achieve a complete picture of the evolutionary history of the family.

### 5OR.9

#### PHYLOGENOMICS OF THE RED ALGAL ORDER NEMALIALES

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The order Nemaliales is part of the highly diverse red algae subclass Nemaliophycidae. Distributed from subantarctic to all tropical seas it comprises both calcified (in most genera of the Liagoraceae *sensu lato* and the Galaxauraceae) and uncalcified (Scinaiaceae) taxa. These features make it a great model to study their evolutionary dynamics. Despite significant improvements in the higher-level systematics and the proposal of several new genera and families in recent years based on analyses of morphology and one or two genes, the phylogenetic relationships among families and genera are not fully explored. We have approached this with high-throughput sequencing of organelle

genomes. Using Illumina Hi-Seq technology we sequenced the entire chloroplast genome of representatives of most genera in the Nemaliales, and extracted >150 genes concatenated in a >120k nucleotide alignment. Our preliminary ML phylogeny recovered a fully resolved tree with very high bootstrap values at all nodes. The data show that the calcified Galaxauraceae family is more closely related to the exclusively uncalcified Scinaiaceae than to the Liagoraceae *sensu lato* that also has calcified species. I will present insights into the evolution of the Nemaliales and explore their radiation in the light of earth history.

### 5OR.10

#### SECONDARY STRUCTURE ALIGNMENT AND MULTIPLE OUTGROUPS CONFIRM THE MONOPHYLY OF THE DIATOM CLASSES USING SSU RNA GENES

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Since 1993, many trees have been produced to infer the phylogeny of the diatoms. The resolution of the monophyly of the three diatom classes described by Medlin and Kaczmarska in 2004 has been controversial. Medlin and Kaczmarska advocated that the monophyly of the three diatom classes could only be achieved if: 1) a secondary structure of the SSU rRNA gene was used to construct the alignment and not an alignment based on primary structure and 2) multiple outgroups. The evidence for both of these factors in recovering the three clades at the class level is evaluated and reviewed. The use of the secondary structure for the alignment for the SSU rRNA gene seems to be accepted but the use of multiple outgroups has only recently been tested. Taking only bolidophytes or other heterokonts as the only outgroups never produced monophyletic clades in single gene analysis but will recover a monophyletic mediophyte class in multi-gene analysis using BI or ML but not using a MP analysis unless it is a weighted analysis. Multiple outgroups including many heterokonts and certain members of the crown group radiation recovered monophyletic classes in single gene and multi-gene analyses. The three classes can be defined by clear morphological differences primarily based on auxospore ontogeny and envelope structure, the presence or absence of a structure (tube process or sternum) associated with the annulus and the presence of the cribrum in those genera with loculate areolae are supported by molecular analyses if the proper conditions are used.



## 6. Symbiodinium as a model organism

### 6KN.1

#### USING *SYMBIODINIUM* GENOMICS TO INFORM CORAL ALGAE SYMBIOSES

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Dinoflagellates are ubiquitous marine and freshwater protists. As free-living photosynthetic plankton, they account for ~50% of the primary productivity of oceans and lakes. As photosynthetic symbionts, they provide essential nutrients to corals, the architects of the ecologically and economically hugely important reef ecosystems. Unfortunately, the molecular mechanisms underlying this partnership are still not well understood. In an effort to better understand coral-algal symbioses, we are currently interrogating several lines of research. In an effort to decoding structure and function of the coral metaorganism, we are completing sequencing, assembly, and annotation of a coral hologenome. The availability of the complete gene set encoded in the genomes of a coral animal, its dinoflagellate symbiont, and the associated bacteria promises an unprecedented view on the composition, structure, and function of the coral holobiont. We are also applying deep-transcriptome sequencing to multiple *Symbiodinium* species at different taxonomic resolutions (i.e. within and between Clades and species) to assess transcriptomic and expression differences at orthologous genes. Further, to better understand the association between stress tolerance of corals and molecular diversity of algal symbionts, we have developed a next-generation sequencing pipeline targeting the ITS2 rRNA gene from *Symbiodinium*. Besides phylogeny-based analyses, we developed cutoffs to delineate *Symbiodinium* diversity in an OTU-based framework. This allows us to compare ocean basins and coral genera in a standardized manner. The presentation will highlight results from these efforts and provide an overview over remaining challenges in establishing *Symbiodinium* as a model organism for marine invertebrate symbioses.

### 6KN.2

#### GENETICS-BASED SYSTEMATICS AND TAXONOMY THRUSTS *SYMBIODINIUM* (DINOFLAGELLATE) RESEARCH INTO THE 21ST CENTURY

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Dinoflagellates in the Genus *Symbiodinium* have become one of the most studied micro-algal groups in science for two reasons. Their mutualisms with animals form the basis of coral reef ecosystems, which are threatened by climate change; and because of the many exciting revelations about their diversity, ecology and evolution gained from the use of molecular-genetic techniques. Currently, however, most researchers studying animal-dinoflagellate symbioses are working and publishing without a well-established taxonomy of these symbionts. This has stalled basic research by reducing the accuracy of scientific inference, prevented comparisons between old and new findings, and has thus hampered formulation of fresh research questions. With incorporation of phylogenetic and population genetic evidence, in combination with available ecological and physiological data, formal species designations are emerging; as the use of ‘clades’ and ‘types’ is slowly discontinued. The underpinning of *Symbiodinium* taxonomy by a sound systematic framework opens numerous research possibilities, including their use as models for the study of eukaryotic microbial dispersal, sexual recombination, adaptation and speciation. The ability to resolve the prevalence and distribution of individual strains (within each species), which are usually homogenous inside a host colony, will lead to the evaluation of inter-individual variation and its importance the response of populations to natural selection.

### 6KN.3

#### *SYMBIODINIUM* - THE FIRST DINOFLAGELLATE KNOWN TO DRIVE MICROBIAL-ALGAL CALCIFICATION

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Dinoflagellates of the genus *Symbiodinium* are best known as endosymbionts of a wide range of marine invertebrates, most notably corals. Their symbiosis with corals, particularly with reef-building species is of central importance for the entire coral reef ecosystem because these microalgae are important primary producers that fuel their hosts' energy requirements, and they enhance coral calcification. However, besides the symbiotic life style, *Symbiodinium* is also capable of living *ex hospite* as a free-living form. These free-living populations represent an important pool for the horizontal acquisition of symbionts by coral juveniles and could play a pivotal role in maintaining coral functional diversity and reef ecosystem viability and yet their biology and ecology remain largely unexplored. Here we show that free-living *Symbiodinium* spp. in culture commonly form calcifying bacterial-algal biofilms with naturally associated bacteria and produce aragonitic spherulites that encase the dinoflagellates as endolithic cells. We provide a description of these novel calcifying biofilms and the produced organomineral. Further, we demonstrate that this calcification is driven by algal photosynthesis but that bacterial communities also critically influence the process. Another important finding was that *Symbiodinium* has the ability to again vacate the formed structures, suggesting that the formation of these microbialites is not an ecological dead-end but instead could comprise part of a temporary endolithic life-cycle strategy. This is the first reported case of a dinoflagellate being the phototrophic driver in a calcifying microbial-algal biofilm, which demonstrates the need to re-evaluate the role of dinoflagellates in organomineralization. Further, our findings provide evidence for an endolithic phase in the *Symbiodinium* life history, a phenomenon that may offer new perspectives on fundamental questions regarding the biology and ecology of these dinoflagellates and may also help explain how changes in ocean chemistry led *Symbiodinium* to ultimately establish an endosymbiotic life style.

#### 6KN.4

### INORGANIC CARBON SCARCITY AND LIMITATION OF *SYMBIODINIUM* IN HOSPITE: ENVIRONMENTAL LIMITATION OR HOST CONTROL

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When engaged in a symbiotic association, the host organism must supply inorganic carbon (Ci) for *Symbiodinium* photosynthesis, while *Symbiodinium* in turn exports reduced organic carbon to the host. We have been studying Ci supply and processing in coral-*Symbiodinium* associations by taking a novel methodological approach that combines physiological tools developed in the microalgal field and modeling. Through work on three species of Caribbean corals, we have found that ambient Ci concentrations limit photosynthesis in 2 out of 3 species, and Ci is scarce inside the host in all species tested. *Symbiodinium* isolated from these hosts show signs of Ci scarcity including high carbonic anhydrase activity and a high photosynthetic affinity for Ci. Despite this apparent scarcity *in-hospite*, the host has highly developed systems for Ci supply and Ci concentrations are high in seawater. We suggest that the host coral may intentionally limit Ci supply as a way to regulate the rate of photosynthesis over short timescales. More speculatively, Ci could be a suitable unit of exchange to ensure that the mutualistic relationship is consistently respected between the partners, since Ci needs to be supplied continuously to the algae and is a precursor of the product desired by the host (organic carbon).

#### 6OR.1

### GENOME ANALYSIS OF TWO *SYMBIODINIUM* ISOLATES

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*Symbiodinium* are symbiotic dinoflagellates that commonly reside in cnidarians *e.g.* corals and jellyfish. A sustained loss of symbiosis between *Symbiodinium* and corals results in coral bleaching and eventual death. To help guide risk management of reef systems in light of climate change, the *Sea-quence* project of the Reef Future Genomics (ReFuGe) 2020 Consortium aims to generate reference genetic data for reef systems at the Great Barrier Reef and the Red Sea. Here we report *de novo* genome assemblies (coverage >30-fold) from two *Symbiodinium* isolates of Clades C (origin: Magnetic Island, Australia) and F (Australian National Algae Culture Collection CS-156), and assess the extent of divergence of these genomes relative to other clades and to other eukaryotes. Genome size is computationally estimated at 1.5Gbp (Clade C) and 1.1 Gbp (Clade F), with overall G+C content ~44%. Major phylogenetic markers of nuclear (*nr28S*, *elf2*) and organellar genes (*cob*, *col*, *psbA*, *cp23S*) were recovered. Of the 458 conserved eukaryote proteins across six model organisms (in CEGMA), we recovered 176 (38%) in Clade C, 149 (33%) in F; the corresponding numbers are higher at 295 (64%) and 267 (58%) when proteins of the chlorophyte *Chlamydomonas reinhardtii* and apicomplexan *Toxoplasma gondii* were included in the analysis. This observation suggests high divergence between genomes of *Symbiodinium* and other eukaryotes. Genome mapping using published transcriptomes (of clades A through D) shows substantial divergence among different clades, with mapped coding regions having higher G+C content (~50%). Our findings support the established inter-clade phylogenetic relationships of *Symbiodinium*, and suggest a trend of genome reduction from the basal lineage (Clade A; estimated size ~3Gbp) through to the more recently diverged lineages of Clades C and F. These genome data provide an excellent analysis platform for testing numerous biological hypotheses related to algal evolution and adaptation.

## 6OR.2

### **SYMBIODINIUM THERMOPHILUM SP. NOV., A THERMOTOLERANT SYMBIOTIC ALGA PREVALENT IN CORALS OF THE WORLD'S HOTTEST SEA, THE PERSIAN/ARABIAN GULF**

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We have recently shown that *Symbiodinium* from the nuclear ribosomal ITS2-type (clade) C3 is the prevalent symbiont in six common coral species along the coast of Abu Dhabi (UAE) accounting for nearly 60% of the analysed ITS2 sequences examined. In contrast, clade D zooxanthellae were not detected from corals in this region. Clade C symbionts have not usually been found to be exceptionally thermotolerant, but have rather been defined as a cosmopolitan, generalist clade. However, recent advances in phylotyping of zooxanthellae using chloroplast sequences (*psbA<sup>ncr</sup>*) suggest that several lineages within 'clade C' represent distinct *Symbiodinium* species. Through analysis of *psbA<sup>ncr</sup>* sequences of C3-type zooxanthellae along >1000 km of coastline in the Persian/Arabian Gulf and the Gulf of Oman we propose that the ITS2-type C3 zooxanthellae from Gulf corals represent a new species. In addition, monitoring the seasonal dominance of zooxanthellae types in individual colonies of six coral species over three years we demonstrate that despite some species-specific variability in seasonal composition of the zooxanthellae complement, this species remains the prevalent symbiont of corals from the Southern Gulf, the world's hottest sea.

## 6OR.3

### **TURNING UP THE HEAT ON SYMBIODINIUM CELL CYCLE ANALYSIS**

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Dinoflagellates of the genus *Symbiodinium* are tied to an endosymbiotic life style with reef forming corals, but through a transient free-living life history phase. Cell cycle as an endosymbiont is controlled by the host coral and typically arrested in the G1 phase (preparation for DNA replication) whereas when in free-living (cultured) *Symbiodinium* cycle across G1 to S (DNA synthesis) to G2/M phases (pre-mitotic gap and mitosis) over 24 h, in regulation of cell proliferation. However, how this cell cycle is potentially influenced by stressors is unknown and represents a key uncertainty as to how this organism can respond to environmental change. Heat stress in particular impacts symbiont fitness but how it impacts upon life history dynamics is as yet unresolved. We therefore initially optimised a protocol suitable for cell cycle analysis of *Symbiodinium*, which was then applied to a large representation of *Symbiodinium* ITS2 type (clades A, B, C, D, and F) and hence account for any possible role of phylogeny upon subtle variations in cell cycle progression. This exercise demonstrated that there were differences in DNA histogram forms across the strains, for example differences in G2/G1 ratios, coefficient variations of G1 peak and the abundances of S and G2/M phase. We subsequently performed a heat stress experiment on a subset of cultures spanning 5 clades, which showed the reduction of cell cycle progression throughout the day and did not show the diel change of the phase fractions. We discuss how this observation particularly impacts *Symbiodinium* cell proliferation through ecological fitness in nature.

#### 6OR.4

##### HISTONE MODIFICATIONS DURING REVERSIBLE TRANSITIONS BETWEEN MOTILE AND NON-MOTILE STAGES OF DINOFLAGELLATES

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Differentiation between motile stages (MS) and non-motile stages (NMS) are common transitions in the life history of dinoflagellates, including the formation of coccoid stage within symbiotic hosts. Dinoflagellates have some of the largest known genomes in the eukaryotes but counter-intuitively have the lowest known chromosomal-to-protein ratios and nucleosomes could not be detected in their chromosomes by conventional assays. It is only recently that genomic studies revealed the presence of dinoflagellate core histones (d-histones) and nucleosome-assembly factors in dinoflagellates, amid very low expression levels. We were able to assemble nucleosomes *in vitro* and CHIP assays also suggested nucleosome formation *in vivo*. We developed large-scale preparations of coccoid stage from a heterotrophic dinoflagellate as a model system to investigate the histone modifications during NMS to MS transition. We observed differential expression in the d-histone proteins, and specific modifications of d-histones are associated with the NMS-MS transitions. Preliminary CHIP-PCR experiments also suggested that d-histones are predominantly associated with actively transcribing genomic regions.

#### 6OR.5

##### THE BIOGEOCHEMISTRY OF SYMBIODINIUM FUNCTION AND COMPETITION

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With one of the largest known eukaryotic genomes, *Symbiodinium* possess the genetic resources for an impressive portfolio of biochemistries. Many of these blueprints code for essential parts to nitrogen metabolism. This may be a result of the ocean's relative oligotrophy over deep time. Today, however, global conditions are rapidly changing and so too has the relationship between *Symbiodinium* and their invertebrate hosts. In this talk, I will present several lines of evidence from eco-physiological and stable isotope investigations that show: 1) that *Symbiodinium* carbon and nitrogen assimilation rates are sensitive to temperature, 2) that different clades/species have competitive advantages for resource acquisition, and 3) that across the diversity of host taxa, the predominant currency of symbiosis is growth-limiting nitrogen. Taken together, these observations support the hypothesis that *Symbiodinium* mutualisms evolved to overcome severe nutrient limitation in the tropical seas.



**6OR.6****LOCAL ADAPTATION OF *SYMBIODINIUM THERMOPHILUM*-ASSOCIATED CORALS TO THE EXTREME ENVIRONMENT OF THE PERSIAN/ARABIAN GULF**

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*Symbiodinium thermophilum* is the prevalent coral symbiont in the southern Persian/Arabian Gulf (PAG). This recently described species represents a promising model for heat tolerant coral symbionts since PAG corals associated with *S. thermophilum* endure summer temperatures of up to 35 degrees Celcius on a regular basis. However, *Symbiodinium*-coral associations in the PAG cope also with the highest salinity levels (often 42-44) reported globally for coral reef ecosystems. The elevated salinity of PAG waters results from the restricted water exchange with the adjacent Gulf of Oman and the wider Indian Ocean, shallow depths, high evaporation rates and limited freshwater input. The resulting halocline circulation is sustained by a net inflow of surface water into the PAG and limited outflow of heavier, more saline bottom waters. While it is well established that local adaptations to prevailing environmental conditions shape the distribution of many terrestrial plants, it is unknown to which extend adaptations, for instance to high local salinity, affect also the spread of algal coral symbionts. Here we demonstrate that local adaptations of *Symbiodinium thermophilum*-associated PAG corals shape the heat stress tolerance of this symbiotic partnership. I will discuss our findings and their implications for coral reef survival and management under the pressure of climate change.

**6OR.7****PHYSIOLOGICAL RESPONSE OF *SYMBIODINIUM* POPULATIONS ADAPTED TO DIFFERENT THERMAL REGIMES IN THE RED SEA**

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Climatic change is causing dramatic changes to the stability of coral reefs worldwide. Studies have particularly focused on the distribution and physiological flexibility of coral-algal symbioses over latitudinal gradients in an attempt to understand how coral reefs will potentially respond to the continued stress caused by climate change. It is clear that corals can flexibly associate with stress tolerant symbionts (genus *Symbiodinium*) when persistently living in high temperature environments or during times of anomalous thermal stress; however, the physiological response of coral-algal symbioses over latitudinal gradients and the very different thermal regimes throughout the largely understudied region of the Red Sea is still unknown. We therefore contrasted the thermal tolerances of coral-algal symbiosis in Thuwal, Saudi Arabia versus Hurghada, Egypt where the seasonal thermal maximum thresholds are very different (32°C and 29°C, respectively). The abundant coral species *Pocillopora damicornis* was particularly interesting because thermal stability was different between populations inhabiting the two locations despite associating with the same clade C ITS2 symbiont: specifically, heat-stress induced declines of photochemical operation was only observed from the naturally warmer more southerly red sea site (Saudi). These physiological differences reveal the importance of adaptation to local environmental conditions yet contradict previous studies that show populations of the same symbiont type living at high temperatures are better adapted to thermal stress. As such, this symbiosis may be living near its thermal maximum on the reefs near KAUST and/or there may be further genetic differentiation for the host and symbiont. Further field surveys at five sites along Egyptian coast from the northern Gulf of Aqaba to southern Egypt (1000 km) are discussed and reveal species-specific differences in coral/symbiont specificity over this latitudinal range.

**6OR.8****NANOSIMS ISOTOPIC IMAGING - DECIPHERING THE NATURE OF CORAL-SYMBIONT METABOLIC INTERACTIONS**

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The ability of endosymbiotic *Symbiodinium* to fix inorganic nutrients and their translocation to the coral host is considered a key element for the growth of coral reefs in tropical coastal waters and has been a fundamental research topic for over 50 years. With pulse-chase experiments using stable isotopes and combined TEM and NanoSIMS ultrastructural and isotopic analyses it is now possible to visualize and quantify the fixation, translocation, and turnover of essential elements such as carbon and nitrogen with unprecedented subcellular resolution. With these techniques we have monitored the assimilation of <sup>15</sup>N nitrate, <sup>15</sup>N ammonium, and <sup>13</sup>C bicarbonate in the coral *Pocillopora damicornis*. These studies have revealed dynamic intra-symbiont storage of assimilated nitrate and ammonium in the form of uric acid crystals, and the formation of lipid droplets and starch granules in the symbiont and their subsequent remobilization. Carbon translocation toward the coral gastrodermal lipid droplets was detected within 15 min. Moreover, glycogen granules in the coral tissue were found to be an important sink for translocated carbon. This work has now been extended to the related pocilloporid species, *Stylophora pistillata*, with the important addition of heterotrophy (feeding the coral with dual-labelled brine shrimps) to contrast the fate and timeframes of heterotrophic vs. autotrophic (<sup>15</sup>N nitrate/<sup>13</sup>C bicarbonate) nutrient uptake under natural conditions and environmental stress due to elevated water temperatures. The *in situ* spatial data provided by NanoSIMS allow us for the first time to address questions that cannot be answered by traditional bulk measurements, such as: (1) the influence of local symbiont densities on individual *Symbiodinium* assimilation rates, (2) metabolic capabilities of different symbiont ITS2 types *in hospite*, and (3) the effect of changes in intracellular host and symbiosome pH on individual symbiont productivity.

## 6OR.9

### CRITICAL ROLE OF OXYGEN PHOTOREDUCTION DOWNSTREAM OF PSI IN SYMBIODINIUM: PHOTOPROTECTION, ENERGETIC ADJUSTEMENT AND ROS PRODUCTION

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The ecological success of symbiotic cnidarians (reef building-corals and sea anemones) relies on the symbiosis between cnidarians and photosynthetic dinoflagellates of the genus *Symbiodinium*. Photosynthetic organisms have evolved various photoprotective and regulatory mechanisms to cope with changing and high light intensities, but the nature and relative amplitude of these mechanisms is still a matter of debate in *Symbiodinium*. Few studies showed that molecular oxygen (O<sub>2</sub>) can be an efficient electron sink during photosynthesis in *Symbiodinium*, with an O<sub>2</sub> uptake capacity that could represent up to half the maximum O<sub>2</sub> evolution. In addition, members of clade A *Symbiodinium* were proposed to possess enhanced capabilities for alternative photosynthetic electron flows. In this work, the amplitude of photosynthetic alternative electron flows to oxygen (chlororespiration, Mehler reaction, mitochondrial respiration) and PSI cyclic electron flow was investigated in *Symbiodinium* strains belonging to different Clades (A, B and F). Joint measurements of oxygen evolution, PSI and PSII activities allowed us to demonstrate that photoreduction of oxygen downstream PSI by the so-called Mehler reaction is the main alternative electron sink at the onset and steady state of photosynthesis in all strains<sup>1</sup>. This mechanism in *Symbiodinium* sustains significant photosynthetic electron flux under high light, thus acting as a photoprotective mechanism and modifying the ratio of ATP/NADPH to match the requirements of carbon reduction. At higher temperature (26 to 33°C), the amplitude of Mehler reaction was still significantly increased while the capacity of enzymes responsible for superoxide detoxification largely decreased. This imbalance generated twice more ROS than during the treatment at 26°C, suggesting that under conditions known to induce coral bleaching, the photoprotective role of Mehler reaction can no longer be maintained, at least at short term.

## 6OR.10

### METABOLIC RESPONSES TO CHRONIC NUTRIENT STRESS DIFFER BETWEEN SYMBIODINIUM PHYLOTYPES

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Dinoflagellates of the genus *Symbiodinium* are a diverse group of microalgae that play a critical role in the energetics of coral reefs. Here, we report differences between two *Symbiodinium* phylotypes (ITS2-types A20 and A13) in their responses to chronic nutrient stress. During a five-week experiment in which batch cultures were grown in initially nutrient-replete (f/2 media; 882  $\mu\text{M}$   $\text{NO}_3^-$ , 36.2  $\mu\text{M}$   $\text{PO}_4^{2-}$ ) or low-nutrient media (22.1  $\mu\text{M}$   $\text{NO}_3^-$ , 0.9  $\mu\text{M}$   $\text{PO}_4^{2-}$ ), we observed divergent cellular stress responses. Photosystem II (PSII) efficiency declined for both types, but did so earlier (by 3-5 days) in type A13. Non-photochemical quenching (NPQ) of chlorophyll *a* fluorescence increased correspondingly, and was more dramatic in type A20. However, these increases were transient as NPQ collapsed following prolonged exposure (> 3 weeks) to low-nutrient conditions. PSII electron transport rate

increased in both types and was driven by increasing PSII functional absorption cross section. Changes in autotrophic capacity (measured as  $^{14}\text{C}$ -labelled bicarbonate-fixation) mirrored those of PSII efficiency, with  $^{14}\text{C}$ -fixation rates declining more dramatically in response to nutrient limitation in *Symbiodinium* A13. Changes in mitochondrial activity (quantified as the activity of citrate synthase [CS], the key “gatekeeper” enzyme of the citric acid cycle) and reactive nitrogen and -oxygen generation (RNS and ROS, respectively) also differed between the two phylotypes. CS activity generally declined following culture inoculation, but then increased in type A20 under nitrate- and phosphate starvation. In type A13, CS activity remained relatively unchanged. Cellular RNS content – quantified using flow cytometry – increased 4-fold in both types under low-nutrient conditions, but significant ROS synthesis was seen only in type A20. These results will be discussed in the context of the dynamic stability of coral-*Symbiodinium* associations, in which the partitioning of fixed carbon, as well as RNS- and ROS synthesis, play key roles while *Symbiodinium* remain in unbalanced growth.

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## 7. Molecular Cell Biology

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### 7KN.1

#### UNEXPECTED DIVERSITY OF CRYPTOCHROME PHOTORECEPTORS FROM ALGAE

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Cryptochromes act as sensory blue light receptors in bacteria, fungi, plants, and insects. They non-covalently bind flavin as a chromophore. In algae, the roles of several different cryptochromes have been identified in the recent years. Some of these cryptochromes group to established subfamilies such as CPH1 from *Chlamydomonas reinhardtii*, a member of the plant cryptochromes. Others are distinguished by their position in the phylogenetic tree or by their molecular properties. The animal-like cryptochrome (aCRY) from *C. reinhardtii* has been demonstrated to respond to yellow and red light as well as to blue light *in vivo*, which expands the role of cryptochromes as blue light receptors to the other regions of the visible spectrum. Moreover, the cryptochrome CryP from *Phaeodactylum tricorutum* is a member of a new subfamily of cryptochromes. The response of algal cryptochromes to light differs also *in vitro* and has been studied by electronic and vibrational spectroscopy. Different redox states of flavin are involved in the photoreactions. In CPH1, a reaction from oxidized to neutral radical takes place. In contrast, aCRY has been postulated to undergo a reaction from the neutral radical to the fully reduced state *in vivo* [Beel et al. *Plant Cell*, 24, 2992.]. This model is in agreement with a change in conformation of the receptor induced by this photoreaction only [Spexard et al. 2014, *Biochemistry* 53, 1041.]. CryP forms a stable radical state of flavin in the dark, which has implications for its role as a receptor in diatoms [Juhas et al. 2014, *FEBS J.* 281, 2299].

### 7KN.2

#### THE *CHLAMYDOMONAS* FLAGELLUM AS A MODEL FOR HUMAN DISEASE

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Cilia and eukaryotic flagella (terms here used interchangeably) occur in organisms ranging from unicellular protists to humans, where they are present in many tissues. *Chlamydomonas* has numerous advantages that have made it a favorite model system for investigating these important cell organelles. Because cilia have been highly conserved throughout evolution, studies of the *Chlamydomonas* flagellum have contributed greatly to our knowledge of the basic biology of ciliary assembly and function. More recently, *Chlamydomonas* has provided important insights into the role of cilia in human health and disease, including primary ciliary dyskinesia, cystic kidney disease, blindness, and hydrocephalus. Cilia are built by a process called intraflagellar transport (IFT), which was first observed in *Chlamydomonas* and involves the active movement of large protein complexes from the base to the tip of the flagellum and then back to the cell body. These complexes carry cargos necessary for the assembly and maintenance of cilia. Studies of IFT in *Chlamydomonas* led to the discovery that polycystic kidney disease, the most common life-threatening inherited disease in humans, is most often caused by defects in receptors displayed on non-motile primary cilia, which function as cellular antennae receiving signals from the environment and relaying those signals back to the cell body. Other human disorders, including blindness and another cystic kidney disease, nephronophthisis, are caused by defects in conserved proteins located at the ciliary transition zone, a little-studied region between the ciliary basal body and ciliary shaft. Studies of these proteins in *Chlamydomonas* have revealed that the transition zone functions as a selective “flagellar pore” or gate that keeps cell body proteins out of the cilium while allowing ciliary proteins into the cilium.

### 7KN.3

#### GREEN ALGAE DIVIDING BY MULTIPLE FISSION – POTENT TOOL (NOT ONLY) FOR CELL CYCLE STUDIES

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Green algae dividing by multiple fission are a diverse group formed by species from unrelated genera. They are connected by a common feature: mother cell under optimal growth conditions divides into more than two daughter cells. This organization of cell cycle represents a potent evolutionary strategy allowing them to exploit maximum of sun light during day for growth without constraints from the cell cycle related processes. The best developed model organism is *Chlamydomonas reinhardtii* but other representatives include biotechnologically important algae such as *Chlorella* sp. and *Scenedesmus* sp. Here, we will summarize the principles governing the cell cycle regulation in algae dividing by multiple fission. Further, we will present an example of possible exploitation of the cell cycle mutants for the study of coordination of cell cycle and response to DNA damage. This work was supported by the GA CR (grant no. 15-09231S), CAS (grant no. M200201205) and by projects Algaman and Algain.

#### 7OR.1

### CELL-TYPE SPECIFIC PHOTORECEPTORS AND ASSOCIATED LIGHT-SIGNALING PATHWAYS IN THE MULTICELLULAR ALGA *VOLVOX CARTERI*

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Photosynthetic organisms, e.g., plants including green algae, use a sophisticated light-sensing system, composed of primary photoreceptors and additional downstream signaling components, to monitor changes in the ambient light environment towards adjust their growth and development. Although a variety of cellular processes, e.g., initiation of cleavage division and final cellular differentiation, have been shown to be light-regulated in the green alga *Volvox carteri*, little is known about the underlying light perception and signaling pathways. This multicellular alga possesses at least 13 photoreceptors, i.e., one phototropin (VcPhot), one UV-B photoreceptor (VcUVR8), four cryptochromes (VcCRYa, VcCRYp, VcCRYd1 and VcCRYd2) and seven members of rhodopsin-like photoreceptors (VR1, VChR1, VChR2, VcHKR1, VcHKR2, VcHKR3 and VcHKR4), which display distinct light-dependent chemical processes based on their protein architectures and associated chromophores. The study of *Volvox* photoreceptors was almost always accompanied by questions regarding their cell-type specific functions, because they are mostly expressed in a cell-type

specific manner. This gives reason to believe that transcriptome pattern of each cell type could change differentially in response to environmental light. Comparison of the gene expression profiles of the reproductive and somatic cells reported revealed that distinct cell-type specific light signaling pathways underlying gene expression modulate appropriate transcript regulation in response to light. Blue light tends to be effective to accumulate transcripts in the somatic cells; while red light leads to accumulate transcripts predominantly in the reproductive cells. Thus, the data show that each cell type has its own genetically predefined light signaling pathways to modulate expression of genes involved in various cellular and metabolic processes including circadian rhythms and photosynthesis in response to environmental light.

#### 7OR.2

### THE CIRCADIAN CLOCK IN THE DIATOM *PHAEODACTYLUM TRICORNUTUM*

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The majority of living organisms evolved circadian clocks, sophisticated mechanisms that synchronize cells with the environmental periodic cues. Although well studied in terrestrial plants and animals, only a few examples of circadian clocks have been described in marine organisms. Diatoms have exceptional adaptation capacities and exhibit circadian behaviors in natural environments. Interestingly, no orthologous of known circadian clock components have been found in the sequenced genomes, suggesting the existence of a diatom specific clock. In order to reconstruct diatom timing mechanisms, we completed high-resolution physiological and transcriptomic analyses of *Phaeodactylum tricornutum* cells grown in diurnal light cycles and free-running conditions. We identified



24 genes showing robust rhythmic expression upon the different treatments, including putative clock inputs, oscillators and outputs, demonstrating a circadian regulation of transcription. Using a mathematical approach, we selected the most rhythmic Transcription Factors (TFs) and explored their function by gene knockdown and over-expression. This allowed the identification of a first clock component, a bHLH-PAS TF, whose deregulation causes growth impairment, loss of circadian gene expression and a negative feedback loop effect on its own transcription. Further characterizations of these and other TF mutants will allow unraveling the circadian clock architecture and its adaptive significance in diatoms.

### 7OR.3

#### DESICCATION TOLERANCE IN STREPTOPHYTIC GREEN ALGAE: NEW INSIGHTS FROM TRANSCRIPTOMICS AND FATTY ACID METHYL ESTER (FAME) ANALYSIS

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Terrestrial green algae frequently experience desiccation. We investigated changes to the transcriptome in Klebsormidiophyceae and fatty acid composition in Zygnematophyceae upon desiccation stress. At ~ 83% relative humidity (RH) the effective quantum yield of photosystem II (Y II, PAM 2500) dropped to zero between 4.5 and 7 hours in *Klebsormidium crenulatum* (SAG 2415). The cells were able to recover 95% of the initial values within 40 min. When desiccated over silica gel (final RH ~10%), Y II dropped to zero within 40-50 min and no immediate recovery of Y II was observed. We analyzed the transcriptome under moist and desiccated (2.5 h silica gel, RH ~ 10%) conditions using RNAseq. The reference transcriptome includes about 24,183 sequences (1.5 million reads, 636 million bases). Over 7000 transcripts changed upon desiccation, 169 transcripts were 10 fold upregulated, including

known factors involved in desiccation tolerance (LEA, ERD), energy production, ROS metabolism and enzymes involved in biosynthesis of raffinose family oligosaccharids (RFO), however many without known function. 330 transcripts were completely suppressed. In *Zygnema* sp. from polar and alpine habitats desiccation tolerance was only observed in mature cultures that form akinetes, in which Y II dropped to zero within 5-7 h at ~83% RH. The transcriptomic analysis in *Zygnema* sp. (SAG 2419) is underway. We analyzed the fatty acid composition in *Zygnema* B, C and E. In all samples the major fatty acids found were oleic and linoleic acid, except in young cultures that also contained palmitic and linolenic acids. We found significant differences in the fatty acid composition between young cultures and all other samples, however hardly any impact of the desiccation/recovery experiment. We conclude that desiccation tolerance occurs in both algal classes, but different mechanisms (i.e. desiccation tolerance only after maturation in *Zygnema*) may be involved.

### 7OR.4

#### THE STRUCTURE AND FUNCTION OF COCCOLITH ASSOCIATED POLYSACCHARIDES: IMPLICATIONS FOR THEIR ROLE IN CALCIFICATION

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Coccolithophores are globally distributed, unicellular marine algae belonging to the phylum Haptophyta. Characterised by internally produced, intricate calcite liths found on the cell surface, they are biogeochemically important due to their role in the transfer of carbon from the upper waters to depth. Research has focused on the mechanisms behind calcification, but exactly how and why they calcify is still unknown. During lith production in the unique Golgi-derived coccolith vesicle, polysaccharides are simultaneously produced and extruded. These coccolith-associated polysaccharides (CAPs) have been shown to play a role in the regulation of calcium carbonate precipitation; and therefore calcification. In this investigation we looked at a range of species of coccolithophores and their CAPs by direct observation and

biochemical analysis. The results show there are clear structural differences between species' CAPs, giving rise to questions of differences in role. Here we present evidence that suggests CAPs are involved in the organisation of the coccosphere, demonstrating that there is more to their function than previously hypothesised.

#### 7OR.5

### CHARACTERISATION OF NUCLEOTIDE TRANSPORTER PROTEINS IN COMPLEX PLASTIDS

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In contrast to land plants, in diatoms the *de novo* biosynthesis of nucleotides takes place in the cytosol, which means that all nucleotides required in the plastids have to be transported across the four membranes surrounding the complex plastid. The genome of the diatom *Phaeodactylum tricornutum* encodes six nucleotide translocator (NTT) isoforms. This unusually high number of NTT genes might enable the transport of newly synthesised nucleotides into the stroma to supply the constant demand for nucleotides, e.g. for DNA- or RNA-synthesis. In previous works, we characterised two diatom NTTs (NTT1 and NTT2), both transporters are most likely targeted into the innermost plastid envelope membrane. NTT1 is a specific adenine nucleotide transporter, which exploits a proton gradient for net import of ATP into the stroma via symport of protons. NTT1 phylogenetically resembles land plant NTTs, which however differ in their transport activity. NTT2 phylogenetically resembles bacterial NTTs and accepts a broad range of substrates (nucleoside triphosphates and their deoxy-forms) which are counter-exchanged against ATP. Here, we present results of the characterisation of the *P. tricornutum* NTT5, which, in contrast to NTT1 and NTT2, is not located in the innermost plastid membrane, but instead is targeted to the outermost or second outermost plastid membrane. Biochemical measurements show that NTT5 apparently acts in an antiport mode and is able to transport a broad substrate spectrum consisting of tri-, di- and mono-phosphorylated adenosine and guanosine nucleotides as well as dATP and dGTP. Such a broad substrate spectrum has not yet been described for any NTT. Comparative sequence analyses indicate that nucleotide transporters resembling NTT5 are exclusively found in diatoms. Taken together, our results show that diatoms apparently depend on a regulated supply of nucleotides not only in the stroma,

but also in the compartments surrounded by the outer plastid membranes.

#### 7OR.6

### INVESTIGATING SIGNAL DOMAINS AND TRANSLOCASES INVOLVED IN PROTEIN IMPORT INTO EUGLENID PLASTID

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Euglenophytes are inner group of euglenids characterized by photoautotrophy and presence of green three-membrane-bound secondary plastid derived from prasinophyte ancestor. Secondary and higher plastids are present in many unrelated protist groups yet their protein targeting seems to have developed in a remarkably convergent way, reflecting the import system already present in the primary plastid and the general endosymbiont-host system features. Components of the TOC/TIC system of the innermost membranes, which are homologous to primary plastid envelope, have been identified in most secondary and higher algae at least on the genomic level. Import across the additional membrane(s) often proceeds through ER or ER-derived pathways.

The presence of targeting sequence in majority of transcripts destined to plastid suggests that TOC/TIC-based system mediates protein transport across the inner membranes of euglenid plastid. Both complexes are probably reduced and/or divergent in primary structure because no component has been identified yet. Import across the third membrane is known to involve vesicles whose recognition and fusion mechanism remains unclear. Using HMM-based algorithm we found *in silico* evidence for Tic21 and Tic32 subunits in *Euglena gracilis* transcriptome. Both subunits were also found in *Eutreptiella gymnastica* (marine species, phylogenetically distant from *E. gracilis*), Tic32 was found in *Euglena longa* (secondarily heterotrophic species closely related to *E. gracilis*) and Tic21 in *Rapaza viridis* (mixotrophic euglenid, sister taxon to all other euglenophytes). Results of further *in silico* analyses of these proteins and localization experiments in *E. gracilis* using specifically designed peptide antibodies will be presented on the conference. We also characterised N-terminal regions of putative plastidal proteins of *E. gracilis* and other euglenophytes mentioned above, namely their length, sequence, hydrophobicity and amino acid composition, in order to design pipeline for prediction of plastid targeting sequences in euglenids.

**7OR.7****FUNCTION OF THE CONTRACTILE VACUOLE IN *CHLAMYDOMONAS*: A SYSTEMS BIOLOGY APPROACH**

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Systems Biology aims to model and discover emergent properties of biological systems. We applied a systems biology approach to osmoregulation and contractile vacuole function in *Chlamydomonas*. We will present a model of CV function/osmoregulation in *Chlamydomonas*. The model incorporates cellular growth as well as osmotic stress and allows prediction of the relative changes in metabolite/protein concentrations upon environmental challenges. The model predicts that only small changes in mRNA/protein abundance are required for adaptation of a *Chlamydomonas* cell to different osmotic conditions. Using quantitative expression analysis and the Illumina RNA-seq platform we tested the predictions of the model experimentally. Transcriptome analyses of *Chlamydomonas* cells were carried out after adaptation to different osmotic stress conditions. We then compared the relative abundances of mRNAs for protein complexes known to be involved in CV function as determined by transcriptome analyses, with the predictions from our model. Generally, experimental and theoretical data agreed very well, however, there are notable exceptions. The relevance of these results for our understanding of osmoregulation/CV function in *Chlamydomonas* will be discussed.

**7OR.8****MOLECULAR REGULATION OF THE DIATOM CELL CYCLE**

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Accounting for approximately twenty percent of the primary production on Earth, the unicellular eukaryotic group of diatoms plays a key ecological and biogeochemical role in our contemporary oceans. Furthermore, diatoms produce various pigments and lipids, and are characterised by their finely ornamented silica cell wall, and therefore they hold great promise for different industrial fields, including

pharmaceuticals, biofuel production and nanotechnology. Despite their major ecological importance and their potential commercial value, many mysteries about the mechanisms that control the diatom cell and life cycle still remain. In particular, their ability to live and dominate in rapidly changing and sometimes harsh environments, suggests that diatoms have evolved specific strategies to adapt to and proliferate in highly fluctuating conditions. Unraveling the regulatory mechanisms that underlie their unique life cycle strategies will be of crucial importance to understand diatom ecology and evolution and to further exploit their industrial potential. Our main research focus is to gain insights into the molecular and genetic mechanisms that control the diatom cell cycle. In this presentation I will highlight our latest findings that demonstrate how diatoms use a common eukaryotic base of cell cycle regulatory components complemented with some novel diatom-specific features to control their cell division, including the role of the diatom-specific cyclin at the light-dependent G1 checkpoint, and the ambiguous cyclin-dependent kinase CDKA2 during mitosis.

**7OR.9****A RECEPTOR-LIKE KINASE, RELATED WITH CELL WALL SENSOR OF HIGHER PLANTS, IS REQUIRED FOR SEXUAL REPRODUCTION IN THE UNICELLULAR CHAROPHYCEAN ALGA, *CLOSTERIUM PERACEROSUM-STRIGOSUM-LITTORALE* COMPLEX**

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Heterothallic strains of *Closterium peracerosum-strigosum-littorale* complex are unicellular charophycean algae, having morphologically indistinguishable two sexes: mating type plus (mt<sup>+</sup>) and mating type minus (mt<sup>-</sup>). Sexual reproduction is easily induced when cells of these two sexes are cultured together in nitrogen-depleted medium in the light. Two glycoproteinaceous sex pheromones (PR-IP and PR-IP Inducer) involved in the progress of sexual reproduction of the *C. psl.* complex have been well characterized. The PR-IP Inducer induces the release of PR-IP from mt<sup>+</sup> cells, whereas PR-IP induces the release of gametic protoplasts from mt<sup>-</sup> cells, during the sexual reproduction. Through the microarray analyses, we identified a gene encoding a novel receptor-like protein kinase and named *CpRLK1*. The *CpRLK1* gene encodes an 1159-aa protein containing an extracellular domain, a

transmembrane domain, and a kinase domain. The CpRLK1 protein was produced after the mixing of both mating-type cells and was also detected only in mt<sup>+</sup> cells in the presence of PR-IP Inducer. In addition, confocal laser microscopy using CpRLK1-specific antibody revealed that CpRLK1 proteins were localized on the conjugation papilla exclusively from one of the paired cells. Analyses of phylogenetic relationships and of extracellular domain organization suggested that CpRLK1 was a member of the CrRLK1L-1 subfamily, which acts as a cell wall integrity sensor in higher plants. Mt<sup>+</sup> cells with knockdown of CpRLK1 showed reduced competence for sexual reproduction and formed an abnormally enlarged conjugation papilla after pairing with mt<sup>-</sup> cells. The knockdown cells were unable to release a protoplast, which is indispensable for zygote formation. We suggest that the CpRLK1 protein is an ancient cell wall sensor that now functions to regulate osmotic pressure in the cell to allow proper protoplast release.

#### 7OR.10

##### UNUSUAL FEATURES OF THE PSEUDOAUTOSOMAL REGION OF A U/V PAIR OF SEX CHROMOSOMES

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The genetic determination of sex requires either non-recombining chromosome regions or complete sex chromosomes, both of which have evolved independently and repeatedly across different eukaryotic species. While the processes shaping the evolution of sex chromosomes are increasingly well understood in diploid organisms, haploid sex determination systems (U/V) have been under-studied. We have recently sequenced and analysed the sex determining region of the brown alga *Ectocarpus*, where sex is expressed in the gametophyte generation, during the haploid phase of the life cycle, and both the female (U) and the male (V) sex chromosomes contain sex-determining regions (SDRs; Ahmed et al. 2014, *Current Biology*). Here, we use experimental and theoretical approaches to investigate the extensive pseudoautosomal regions (PARs) that border the SDR on the *Ectocarpus* sex chromosome. Despite a considerable

amount of theoretical work on PAR genetics and evolution, these genomic regions have remained poorly characterized empirically, even in classic model organisms. We show that although the PARs of the U/V sex chromosomes of *Ectocarpus* recombine at a similar rate to autosomal regions of the genome, they exhibit many features typical of non-recombining regions. The pseudoautosomal regions also preferentially accumulate sporophyte-biased genes, which tend to occur in physically linked clusters. A modelling-based approach was used to investigate possible evolutionary mechanisms underlying this enrichment in sporophyte-biased genes. We provide the first detailed analysis of the recombining regions of a haploid sex chromosome system and propose a mechanism that may explain some of the exceptional evolutionary features of these regions compared with autosomes.

#### 7OR.11

##### CONSERVATION OF THE AUTOPHAGY PATHWAY IN PHYTOPLANKTON AND ITS ROLE DURING PHOSPHOROUS STRESS

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Phytoplankton contributes ~50% of the global photosynthesis and serve as the foundation of marine food webs. Although their eco-physiology is extensively studied, some basic aspects of the algal cell biology remain obscure. The recent wealth of algal genomic resources has opened new frontiers to decipher cellular pathways and their ecological function. Autophagy is a common eukaryotic pathway to recycle unwanted cytoplasmic content via specialized vesicles, serving as a key cellular mechanism against pathogens and nutrient starvation. We performed a genomic analysis of autophagy-related (ATG) proteins in green, red and chromalveolate algae. We elucidate that ATG proteins are conserved among green algae, but intriguingly missing in red algal genomes, suggesting that autophagy is not conserved in red algae as it is in the entire eukaryote domain. However, chromalveolates retain ATG genes, despite being derived from the red plastid lineage, raising fundamental questions regarding the acquisition of ATG genes during algal evolution. Among chromalveolates, *Emiliania huxleyi* (Haptophyta), a bloom-forming coccolithophore, possesses a complete set of ATG genes. In light of this, the role of autophagy in *E. huxleyi* cells was addressed during phosphorous (P) limitation, typical for many aquatic ecosystems. *E. huxleyi* exhibits remarkable physiological abilities during P scarcity, including internal P storage, induction of phosphatases and



transporters. We investigated additional cellular mechanisms that may promote survival of P deficient cells. Upon P-limitation, ATG gene expression was induced, together with formation, acidification and degradation of autophagic vesicles. Moreover, the membrane's lipid composition was remodeled, as ~30% of the phospholipids were substituted with sulfo- and betaine lipids during acclimation to P limitation. Interestingly, the autophagy inhibitor wortmannin reduced phospholipid substitution, proposing a possible interplay between the two processes. These bioinformatic analyses and lab-based experimental findings provide basis for future field experiments regarding the role of autophagy in natural populations in the ocean.

## 7OR.12

### **THE ROLE OF AN ANIMAL-LIKE CRYPTOCHROME IN THE LIFE CYCLE OF THE UNICELLULAR GREEN ALGA *CHLAMYDOMONAS REINHARDTII***

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Cryptochromes (CRYs) are a class of blue-light sensitive flavoproteins found in plants, fungi, microbes and animals. In the green alga *Chlamydomonas reinhardtii*, a plant CRY and an animal-like CRY (aCRY) exist. Interestingly, aCRY acts not only as a blue light-, but also as a yellow and red light-sensory receptor [Beel et al., *Plant Cell*, 2012 (24): 2992-3008; Spexard et al., *Biochemistry*, 2014 (53): 1041-1050]. In *C. reinhardtii*, two steps in its sexual life cycle are controlled by light, gametogenesis and zygote germination [Huang and Beck, *PNAS*, 2003 (100): 6269-6274]. It was shown that gametogenesis depends largely on blue light but also to a small extent on red light [Weissig and Beck, *Plant Physiology*, 1991 (97): 118-121]. Phototropin was shown to be involved in this process but it is not able to perceive red light. Since aCRY is the only so far known photoreceptor that can absorb red light in *C. reinhardtii*, we started to examine if it plays a role in the life cycle of *C. reinhardtii*. Here, we will present data about changing expression levels of aCRY and its role during the life cycle.

## 8. Algal biodiversity and ecosystem function: new scenarios in coastal systems

### 8KN.1

#### THE CONSEQUENCES OF CHANGES IN ALGAL BIODIVERSITY

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The Earth's environment is rapidly changing, mainly due to a growing human population and more diverse activities. Much research has focused on the impact of such environmental changes on biodiversity. There is now accumulating evidence that the opposite relation will also occur: changes in biodiversity will greatly feed back to the environment and ecosystem processes. The first experimental manipulations of biodiversity emerged in the early 1990's, and since then there has been an exponential increase in the number of published studies. Theory predicts that biodiversity can be an important factor influencing how ecosystems work, because species occupy distinct niches, can be complementary in their use of resources, and experience positive interactions. Indeed, communities with a diverse set of species generally produce more biomass and use resources more efficiently, compared to average communities with fewer species.

This talk will give an overview of the current state-of-the-art when it comes to research about the functional consequences of changes in algal biodiversity: what we currently know, and where we need to go next. It will provide a range of examples, from both experimental and observational studies, in freshwater and marine systems.

### 8KN.2

#### STRUCTURE AND DYNAMICS OF THE SOUTHERN RANGE LIMIT OF A CANOPY-FORMING ALGA AND THE CONSEQUENCES FOR THE COASTAL ECOSYSTEM FUNCTIONING

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The loss of canopy-forming algae, such as furoids and kelps, has been documented across many parts of the world, and has often been attributed to climate change and other human related stress factors. A major challenge in ecology is not just to understand and predict changes in the distribution of key species under climate change, but also to identify the impacts of these changes on the ecosystem functioning. The rocky coasts of Iberian Peninsula constitute the southern range limit for a considerable number of canopy-forming, cold-temperate algae, which dominate shores of Northern Europe. Among these species is found the intertidal seaweed *Fucus serratus*. Recent observations have indicated a continuous drastic westward retraction of this species in N Spain, related to ocean warming and other climatic factors linked to emersion times. The local extinction of *F. serratus* has led to changes in the structure of the benthic assemblages and lower trophic positioning of some consumers, which suggests an overall shrinkage of the food chains length. As a consequence of the observed distribution retraction, the presence of *F. serratus* in the NW Iberian Peninsula is nowadays virtually limited to a few scattered but persistent populations, mostly inside large embayments (*rías*) of Galicia. These remnant populations show signs of local adaptation/acclimation and are located in places with more benign environmental conditions than surrounded areas. Their future viability will depend in part on the expected trends of upwelling strength in the area. A recent study reveals a weakening of the Iberian upwelling during the last decades. The retreat of canopy-forming algae seems to be a general phenomenon in the Iberian Peninsula, involving other furoid and kelp species. Under present and future climatic scenarios, local extinctions of coastal ecosystem engineers would entail a reorganization of natural assemblages and the simplification of coastal food-webs.

### 8OR.1

#### CHANGES IN PHYTOPLANKTON COMMUNITY COMPOSITION AFFECT BIOGEOCHEMICAL FLUXES; AN INDIRECT EFFECT OF GLOBAL CHANGE

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The spring bloom in the Baltic Sea is by far the most important phase of the annual succession in terms of primary production. Two phytoplankton groups are predominant during this period: diatoms and dinoflagellates. Climatic changes have caused dinoflagellates to become more dominant at the expense of diatoms in parts of the Baltic Sea. We investigated the effects of phytoplankton community composition on spring bloom carbon flows and nutrient stoichiometry in multi-year mesocosm experiments. Comparison of differing communities revealed that community structure significantly affected C accumulation parameters, with highest particulate organic carbon (POC) build-up and dissolved organic carbon (DOC) release in diatom-dominated communities. In terms of inorganic nutrient drawdown and bloom accumulation phase, the dominating groups behaved as functional surrogates. Dominance patterns, however, significantly affected C:N:P:Chl *a* ratios over the whole bloom event: when diatoms were dominant, these ratios increased compared to dinoflagellate dominance or mixed communities. Diatom-dominated communities sequestered carbon up to 3.6-fold higher than the expectation based on the Redfield ratio, and 2-fold higher compared to dinoflagellate dominance. Furthermore, after the peak of the spring bloom diatoms generally settle quickly to the sea floor causing aggravated oxygen conditions in bottom waters, whereas dinoflagellates either lyse already in the water column or produce resistant resting cysts that do not degrade in the sediment. The dominance by either phytoplankton group thus affects directly both the summertime nutrient pools of the water column, and the input of organic matter to the bottom sediments, to contrasting directions. Our results highlight the need for remote sensing technologies with taxonomical resolution. Climate-driven changes in phytoplankton dominance patterns will have far-reaching consequences for major biogeochemical cycles and need to be considered in climate change scenarios for marine systems.

## 8OR.2

### INFLUENCE OF SEDIMENT BIOFILM 'PHASE OF GROWTH' ON HIGH MICROPHYTOBENTHOS (MPB) VARIABILITY ON AN INTERTIDAL FLAT

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Intertidal microphytobenthos, the community of benthic diatoms and associated other species that dominate many soft-sediment habitats, often exhibit significant levels of spatial variability in biomass. We show that more than 25 % of microphytobenthos variability within Colne Estuary, UK, was due to differences between the replicates (at spatial scale < 0.5 m). A pronounced relationship was revealed between the MPB 'phase of growth' (determined using *chl a* and colloidal carbohydrate concentrations) with species richness, species composition and relative abundance. Nonetheless, relative abundance of the most dominant species *Gyrosigma balticum* and common species *Gyrosigma scalproides*, *Gyrosigma limosum*, *Gyrosigma litorale*, *Diploneis didyma*, *Pleurosigma angulatum* and *Navicula digitoradiata* can be used to imply the MPB biofilm 'phase of growth'. The dominance of these species in the biofilm, however, was reduced by the appearance of centric planktonic species such as *Coscinodiscus* spp and *Actinopterychus splendens* during periods of spring tides. Species richness of lag and early exponential phases, and latter exponential and stationary phases were found to range between 28 to 34 species and 36 to 45 species, respectively. In addition, 'older' biofilm 'phase of growth' had lower Shannon Wiener species diversity than earlier stage biofilm. This study has proved that MPB assemblages, even in a same biofilm mat (at scale < 0.5 m) can have different a 'phase of growth', which strongly depends on the species relative abundance and richness. Also, the age of MPB biofilm showed to control the erosion effect on the MPB community during immersion and the biofilm 'phase of growth' in the following emersion period. Henceforth, the high variability derived from the difference between the replicates, which normally known as 'background noise' is actually a product of different age or 'phase of growth' of the MPB biofilm.

## 8OR.3

### INFLUENCE OF INTERSPECIFIC COMPETITION ON PHOTOSYNTHETIC RATES OF ALGAL COMMUNITIES

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Resource competition is a major determinant for the structure and dynamics of diverse phytoplankton communities. Due to taxon specific functional traits of primary producers, species richness in diverse

communities might be the underlying mechanism mediating biodiversity effects. For the purpose of light harvesting, species rich phytoplankton communities are able to use the different spectral components of the photosynthetic active radiation (PAR) more effectively, related to differences in composition with accessory pigments and light acclimation. We conducted measurements using the Pulse Amplitude Modulation fluorometry (PAM) based on differences in accessory pigments among phytoplankton taxonomic groups as well as fluorescence based chlorophyll a quantification to assess the influence of competitive stress on the photosynthetic performance of selected phytoplankton species. In growth experiments under competitive co-limitation for phosphorus and light we compared the photosynthetic capacity of mixed algae communities, either i) grown together experiencing interspecific competition or ii) pipetted artificial communities out of monocultures experiencing intraspecific competitive stress. We assume that the intra- and interspecific competitive ability of phytoplankton species for light does affect the photosynthetic rates of phytoplankton species. We argue that the light availability and nutrient supply ratio to each phytoplankton cell is directly affected by the competing cells in close vicinity. First results and interpretations respecting the effects of interspecific competition on the photosynthetic capacity on algal communities will be presented.

#### 8OR.4

##### HABITAT CHARACTERISTICS DRIVING SPECIES RICHNESS IN ROCK POOLS

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Species richness is a key aspect of community structure, and can influence the stability and magnitude of ecosystem functions. Often, patterns of species richness are attributed to heterogeneity (i.e., variation in physical and biotic factors) across habitat. However, describing this heterogeneity-richness relationship requires an initial understanding of how species simultaneously respond to a multitude of habitat characteristics generated at different spatial scales (e.g. micro- vs. macro-habitat). Using intertidal rock pools as a model system, we systematically surveyed multiple quadrats within individual pools to differentiate between micro-habitat characteristics which vary within the pool (i.e., surface complexity, depth, slope), and macro-habitat characteristics which pertain to the entire pool (i.e., elevation, volume, grazer abundance). We then applied mixed effects models to quantify the relative importance of each habitat

characteristic in driving both algal species richness and individual species abundance. Prior to analysis, the species *Corallina officinalis*, was of particular interest because it provides hard, complex structure within the algal community, possibly promoting additional algal settlement. We found that aspects of both the micro- (i.e., slope and depth) and macro-habitat (i.e., elevation and grazer abundance) of these pools drive species richness, with effects for each covariate ranging in both sign and strength. *Corallina* abundance appears to be only weakly influenced by a subset of these variables, but its abundance is positively related to overall species richness. Thus, key individuals may contribute to overall patterns of diversity. These results suggest that habitat characteristics, which become biologically relevant at different levels of observation, may differentially influence individual species within the community in addition to species richness as a whole. Therefore, the scales at which these characteristics affect the establishment and survival of various species will collectively dictate the patterns observed in species richness across a heterogeneous habitat.

#### 8OR.5

##### UNCONSCIOUS UNCOUPLING: LIFE CYCLE PLASTICITY FACILITATES MACROALGAL INVASIONS

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Macroalgae comprise nearly 20% of all marine species introduced into non-native habitats and profoundly



alter local detrital and trophic food webs. We propose that the success of macroalgal invaders depends on the flexibility of its biphasic life cycle to respond to local environments. Using 10 microsatellite loci to genotype > 2000 individual thalli from across 62 populations, we found that the haploid-diploid red alga *Gracilaria vermiculophylla* expanded from northern Japan and entered high-salinity, temperate estuaries across three continental margins of the Northern Hemisphere. In its native range, *G. vermiculophylla* is mostly found on intertidal hard substrata and ~60% of individuals are diploid. In contrast, most *G. vermiculophylla* populations in non-native habitats are found on soft-sediments, occurring as drifting mats or anchored by *Diopatra* worms. Accompanying this ecological shift is an uncoupling of the biphasic life cycle in the non-native range. Twenty-four of 35 non-native populations exhibited diploid frequencies of 90%. Eleven of these 24 populations were at diploid fixation. The ecological and evolutionary consequences of the functional loss of free-living stages are poorly understood, as are the mechanisms underlying the evolution and maintenance of biphasic life cycles. Yet, this life-cycle flexibility likely facilitates invasion success of biphasic groups, such as macroalgae and, thereby, represents an important, but poorly described component of global change.

#### 8OR.6

##### MODELLING THE POPULATION DYNAMICS OF INVASIVE *UNDARIA PINNATIFIDA* USING AN INDIVIDUAL-BASED APPROACH

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In this project, an individual-based modelling approach (in association with field studies, ecological experiments and molecular work by the research group) is being taken to study the impact of an important invasive kelp species, *Undaria pinnatifida*, on native biodiversity in European coastal waters under variable climatic conditions. The theory of autonomous agents is a useful approach for the modelling of algae communities as it allows large-scale population models to be derived from simple rules dictating the growth and interactions of the individual members. A detailed model of the life history of *U. pinnatifida* (including both the microscopic and macroscopic stages of its life cycle) has been developed using empirical data from the literature on their responses to various environmental factors (e.g. light, water temperature, day length). This has been

integrated into a spatio-temporal model of a coastal environment in order to make predictions about the potential growth dynamics under different environmental conditions. Comparisons between model predictions and field studies of an invasive population of *U. pinnatifida* in Brest harbour, France, show good quantitative agreement in terms of relative abundances, rates of recruitment and life expectancies of mature sporophytes. Future work will include using the model to make predictions about their potential responses to changing climatic conditions and to assess the risk for future range expansion in European coastal waters.

#### 8OR.7

##### THE INTEGRAL ROLE OF PHAEOPHYCEAE TO DRIVE COASTAL IODINE FLUXES: CASE STUDIES FROM IRELAND

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Biogeochemical processes of iodine in coastal marine systems are largely driven by brown macroalgae. Through exposure to environmental stressors many iodine-rich Phaeophyceae can act as biological source of iodine, influencing not only hydrological and atmospheric chemical processes but also human health. However, the capacity of Phaeophyceae to retain and release iodine remains to be quantified. We have developed a cost-effective HPLC method to measure total iodine contents in macroalgal matrices and investigated the release of algal iodine in relation to the physiological state. Laminariales collected from the Irish west coast contained iodine at levels up to two orders of magnitude higher than Fucales, although considerable intra-thallus and seasonal variation was observed; this suggests an environmental impact on algal iodine metabolism. *Laminaria digitata* emitted large quantities of iodine into the atmosphere immediately following air-exposure; iodine emission rates were enhanced under abiotic stress conditions and appeared to depend on tissue iodine concentration. Under submersion, low salinities caused a decrease in the physiological performance which coincided with increased iodine release into seawater, indicating an osmotic function of iodine. During degradation, algal biomass released and emitted large quantities of iodine into the environment, demonstrating that the biogenic iodine transfer in coastal marine systems is further driven by degrading biomass. Our results emphasise the integral role of temperate Phaeophyceae in the coastal iodine cycle. We provide

evidence that physiological responses and degrading biomass of brown macroalgae are the main vectors of coastal iodine fluxes.

### 8OR.8

#### **BRIDGING THE GAP BETWEEN ALGAL ECOLOGY AND BIOTECHNOLOGY – MORE THAN JUST LEARNING A NEW LANGUAGE**

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The assessment of algal chemical composition has become an important integral component of numerous international research programmes that investigate new sources of high-value compounds. Of particular interest to food, agricultural, pharmaceutical and cosmetics industries are commercially valuable metabolites such as phycobilines, carotenoids, MAAs, fatty acids and phenolic compounds. Fulfilling multiple physiological functions, clearly some compounds vary naturally according to algal species under investigation, location and season, amongst many other factors. Understanding the ecological and metabolic responses of algae (sea-

weeds and microalgae) to their immediate environment (including climate change, harvesting regimes, water quality) is thus fundamental to the future development of algal biotechnology. However, the application of ecological knowledge needs to reach beyond provision of baseline data to industry. With increasing commercial pressures, the sustainable supply of algal biomass for specific applications, which can potentially be sourced from natural or cultivated stocks, becomes critical. This presentation gives an overview of current research aimed at identifying the drivers of observed variability and the application of physiological mechanisms to enhance bioactivity in natural algal biomass (through selective harvesting, or via targeted, optimised biomass production in culture) for safe and stable algal products from sustainable sources. Despite technical advances in the development of analytical methods, the biological pathways that implicate commercial value of algal biomass are still poorly characterised even for the most commonly used algal species; also there are significant gaps in our understanding of chemical diversity and the variability of algal bioactives, and the natural and process-induced factors that control them. Additionally, dialogue with industry is essential to identify specific research needs while ensuring sustainable management of natural resources and preserving ecosystem integrity.

## 9. Algae in stressful environments

### 9KN.1

#### EXTENSIVE GENETIC DIVERSITY AND DIFFERENTIAL BI-ALLELIC EXPRESSION IN A SOUTHERN OCEAN DIATOM

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Despite being one of the most inhospitable regions on Earth, the Southern Ocean houses a highly diverse and productive community of organisms. Unicellular eukaryotic diatoms are the main primary producers in this environment where photosynthesis is limited by low concentrations of dissolved iron, large annual fluctuations in light, temperature and the extent of sea ice. How diatoms have evolved to this extreme environment is largely unknown. Here we present insights into the evolution of a cold-adapted (psychrophilic) diatom from the Southern Ocean, *Fragilariopsis cylindrus*. Our study reveals that the genome of *F. cylindrus* contains highly diverged alleles that are differentially expressed depending on the environmental conditions and stresses imposed. Alleles with largest ratio of replacement over silent substitutions (largest  $d_N/d_S$  ratio) show the most pronounced condition-dependent expression. This suggests that environmentally-induced diversifying selection drives the allelic differentiation. The highly diverged alleles with nucleotide divergence of up to 6% show nevertheless a signature of genetic recombination. Many of the differentiated alleles encode proteins from conserved core and lineage-specific metabolism indicating the requirement to fundamentally adjust metabolism to cope with an extreme and variable environment. These homologs account for 73% of all *F. cylindrus*-specific transcripts in natural sea-ice communities, including the most highly abundant transcripts. In this vast gene pool, allelic variants adapted to particular conditions are maintained, thereby enabling the population to respond to the highly variable environment of the surface Southern Ocean.

### 9KN.2

#### FROM ETERNAL DARK TO HIGH LIGHT STRESS: MICROALGAE IN THE HIGH ARCTIC AND THE CHALLENGE OF DIFFERENT EXTREMES

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At high latitudes, algae encounter extreme light conditions, ranging from months-long darkness during the Polar night, to sudden high-light exposure during ice break-up in spring/ summer, when the sun never sets. At present, very little is known about how microalgae overwinter at latitudes where the sun does not rise for several months. In addition to resting stages in the sea ice or sediment, vegetative cells of numerous species occur in the water column throughout the entire winter (although in extremely low abundances). A recent attempt to describe their physiological state revealed a system that, even in the middle of the Polar night, has the potential to resume photosynthetic production, virtually instantaneously, upon re-illumination. Furthermore, resting stages from sediment surfaces reacted to light exposure after only 6 days – their activity levels, growth rate and primary productivity being dependent on both day length and light intensity. Since algae from sediment, sea ice and seawater habitats likely contribute to the seeding population responsible for the very distinct spring peak in annual productivity after the winter, a comprehensive understanding of physiological adaptations is very important. The bulk of primary production in the high Arctic occurs during this relatively short transition from spring to early summer. As soon as sufficient light becomes available, extremely shade-adapted algae in the bottom layer of sea ice start forming blooms, usually well before their pelagic counterparts build up high biomass. However, during the ice-melting period, optical conditions in the sea ice environment undergo abrupt changes, ending with surface blooms in open water that are exposed to very high irradiances. Such alterations in light conditions have a pronounced effect on algal physiology and biochemical composition, including elemental stoichiometry or essential polyunsaturated fatty acids.

### 9OR.1

#### BIODIVERSITY ASSESSMENT AND TRANSCRIPTOMIC ANALYZES OF EUKARYOTIC SEA ICE DIATOMS OF THE CENTRAL ARCTIC OCEAN

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Sea ice is a large environment controlled by a number of hostile abiotic factors, i.e. low temperatures, low light intensities, and high salinities. Despite these harsh conditions, it is inhabited by a diverse community and significantly contributing to primary production in ice-covered regions. While this biodiversity has been investigated by classical methods in the past, little is known about its functional biodiversity, i.e. which species are actively contributing with which functions to the community. By sequencing 18S rRNA and rDNA amplicons, we found an over-representation of certain groups (i.e. Bacillariophyceae and Ciliophora) in the active part of the community. Furthermore, we were able to isolate an abundant naviculoid sea ice diatom member of the Arctic sea ice community (CCMP2297) and conducted temperature stress experiments (10 °C, 5 °C, -2 °C, -5 °C) to analyze physiological and transcriptional acclimation to high and cold temperature stress. Differential gene expression was investigated using RNASeq with a HiSeq2500 sequencer. We observed that based on physiological parameters this diatom has a broad thermal range (5 °C to -5 °C). Remarkably, stress levels that did not lead to physiological alterations already caused a transcriptomic response. The highest number of differential expressed transcripts was found in response to 5 °C, indicating that this temperature is more harmful to CCMP2297 than low temperatures.

## 9OR.2

### ADAPTATION OF DIATOM EXTRACELLULAR POLYMERIC SUBSTANCE (EPS) PRODUCTION IN RESPONSE TO TEMPERATURE AND SALINITY STRESS IN SEA ICE ENVIRONMENTS

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Abundant and productive assemblages of predominantly pennate diatoms are found living in the networks of brine channels and pores within sea ice. These algae are exposed to steep gradients in conditions across the vertical profile of ice flows, experiencing temperatures as low as minus 20°C and salinity values in excess of 50. One adaptation to these stress conditions is enhanced production of a range of extracellular polymeric substances (EPS, mucilages) that appear to provide a protective function to the cells. Results from culture studies on the diatoms *Cylindrotheca closterium*, and the ice diatoms *Fragilariopsis cylindrus*, *F. curta* and *Synedropsis* sp., reveal that EPS can protect cell growth and photosynthesis from chronic and acute salinity stress, and that EPS production is altered under a matrix of changing conditions of temperature and salinity similar to those experienced in the sea ice environment. These laboratory data are compatible with field observations on EPS concentrations and composition from Arctic and Antarctic sea ice cores, which reveal the existence of consistent patterns in the characteristics of EPS. Sea ice diatoms are the major producers of EPS in sea ice, and these EPS play an additional role in water column processes after seasonal ice melt. EPS have also been implicated in ocean-atmosphere interactions. Thus the physiological responses of diatoms to the stresses of living within the sea ice matrix are connected to larger scale environmental processes.

## 9OR.3

### VEGETATIVE SURVIVAL AND STRESS TOLERANCE OF ZYGNEMA SPP. (ZYGNETOPHYCEAE, STREPTOPHYTA) IN POLAR REGIONS

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Filamentous conjugating green algae (Zygnemato-phyceae) belong to the most common primary producers in the polar hydro-terrestrial environment. In such unstable habitats, organisms are subject to various stress factors, e.g., freeze–thaw cycles, desiccation and high irradiation levels. Polar *Zygnema* spp. were examined under various stress conditions both in the field and in experiments with cultures. At the end of summer, the cells gradually lose their typical appearance (with large vacuoles and stellate chloroplasts) and form pre-akinetes, stationary-phase-like cells. Such modified vegetative cells are filled with lipids and characterized by reduced chloroplast lobes and thickened cell walls. Formation of pre-akinetes in field conditions was not triggered by desiccation, but hardening during slow dehydration was required for the pre-akinetes to become osmotic stress tolerant. In experimental conditions the formation of pre-akinetes was induced by nitrogen starvation. In general, viability and recovery rate after experimental desiccation depended on pre-cultivation conditions and drying rate. The pre-akinetes survived even rapid drying (at 10% relative air humidity) when hardened by mild dehydration stress and viable pre-akinetes were even observed in field conditions during winter. The results indicate that naturally hardened pre-akinetes play a key role in survival under extreme conditions of polar climate, while the production of specialized cells with resistant cell wall (e.g., zygospores) is largely suppressed. Moreover, desiccation-tolerant cells derived from disintegrated filaments can act as air-borne propagules.

#### 9OR.4

##### SNOW ALGAE ALL OVER SVALBARD? - AN (OTHER) ATTEMPT TO EXPLAIN THEIR DISTRIBUTION PATTERNS

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Microscopic algae also thrive on the stressful cold environments of snow and glacier fields in high polar and many high mountain regions of our earth and their blooms become visible as the phenomenon of red and green snow. Though generally abundant in

these areas, their occurrence in a region can vary greatly. Many attempts have been made to decipher the decisive abiotic and biotic factors - often with a focus on a small set of specific factors. In August 2013 we performed a field campaign in the Svalbard archipelago, surrounding the Island of Spitsbergen and parts of Nordaustlandet. Large scale mapping of red, green (and white) snow, microscopic and chemical analyses of field samples, examination of the respective geology, digital mapping of snow and glacier field topography and structure using an unmanned air vehicle (UAV) with visible and thermal infra-red cameras, together with field observations from earlier expeditions and considerations of the climatic conditions led to a rather rough, but conclusive explanation of the distribution patterns of snow algae on Svalbard. No single chemical factor (pH, nutrients), nor field topography and the relating water availability, nor the underlying geological formation was a sole decisive factor promoting snow algae. On a small scale such measurements might explain local snow algal communities, but from a more comprehensive view, one factor such as the main geology, e.g. of carbonate rocks prevailing in a region or the overall extremely dry climatic conditions in a cold desert can dominate negatively over other, rather beneficial factors. This presentation will present data from the field campaigns and will try to convey this rather integrative approach to explain the occurrence of these very interesting extremophiles.

#### 9OR.5

##### CYANOBACTERIA AND MICROBIAL MATS IN ANTARCTIC TERRESTRIAL AQUATIC ECOSYSTEMS: DIVERSITY, DISTRIBUTION AND RESPONSE TO ENVIRONMENTAL VARIABLES

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Antarctic terrestrial aquatic ecosystems in the McMurdo Dry Valleys, Southern Victoria Land and

Ross Island are extreme environments because of permanently low temperatures, short growth periods and water chemistries. Cyanobacteria are key primary producers in benthic environments of streams, melt-water ponds and perennially ice-covered lakes, and a major structuring agent of microbial mats. Recent findings have shown the sensitivity of these inland ecosystems to climatic-driven environmental change, and a better understanding of the distribution and response of cyanobacteria and other microbes within cyanobacteria-based microbial mats to environmental stress, will assist in evaluating the resistance and resilience of Antarctic freshwater benthic biology to change.

We evaluated cyanobacterial diversity and microbial mat communities in perennially-ice covered meromictic lakes in the McMurdo Dry Valleys, Antarctica using 16S rRNA gene environmental surveys. These lakes are useful models to study the relationship between microbial communities and environmental variables because they have rich benthic cyanobacterial mat accumulations and stable stratification of physical and chemical conditions. Our analysis suggested that macroscopic morphologies and internal stratifications of cyanobacteria-based microbial mats differed between lakes, and can co-vary with oxygen and light levels, and potentially also be influenced by cyanobacteria taxa composition. However, the majority of cyanobacteria ribotype diversity was shared between lakes, and only a weak relationship between community structure and environmental variables such as conductivity and photosynthetically active radiation and dissolved reactive phosphorus was evident. This suggests that cyanobacteria have the capability to colonise, adapt and grow across broad environmental ranges, and such adaptability may provide a high degree of community resistance and resilience to future climate-driven-environmental change in Antarctic terrestrial aquatic ecosystems.

## 9OR.6

### BENTHIC ALGAE AND CYANOBACTERIA FROM EGYPTIAN DESERT SPRINGS AND WELLS: ISOLATED, STRESSFUL, AND IMPACTED FRESHWATER HABITATS

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Starting as an informal collaboration, the scientific work on the benthic algae of springs and wells of Egyptian Oases has been included in the MUSE (Trento, Italy) research programs as the PhyBiO Project (Phycological Biodiversity in Oases, and the Challenges for its use in Bioassessment of Water Resources). This contribution offers an overview on several phycological investigations carried out to achieve a deeper knowledge of the algal and cyanobacterial biodiversity of Egyptian desert springs and wells. Analyses are based on barcoding and molecular phylogenetic inference, morphotaxonomy, life cycle features, bioorganic screening, and in some cases cytological observations and karyological analysis. The following taxa are being addressed: - two species in the green algal genus *Rhizoclonium* including an Egyptian species new to science, *R. aegyptiacum* Saber et Cantonati sp. nov., and *R. hieroglyphicum* collected in Italy; - a *Stigeoclonium*-like green alga that is most likely a *Caespitella* species new to science (from Ain Helwan); - taxa from the green algal genera *Zygnema* and *Zygnemopsis*; - stoneworts: *Chara vulgaris* from two locations in eastern (Moses Springs, Sinai) and western (Siwa Oasis) Egypt respectively; - *Westiellopsis prolifica* (cyanobacterium); the red algae *Compsopogon* (Egypt, thermal spring)/*Lemanea* (Italy, high-elevation glacial stream), which will include a comparison between the membrane lipids of two red algae species with a similar thallus architecture but from thermally contrasting habitats; - diatoms: preliminary analyses already pointed out species of particular interest in the genera *Achanthidium*, *Halamphora*, *Navicula*, and *Ulnaria*. The environments studied are selective (mineralization, temperature etc.), isolated and thus potentially highly-interesting from the biogeographic point of view but also strongly-impacted due to exploitation by humans because these are the only water resources available in the area.

## 9OR.7

### LIGHT AND NUTRIENT EFFECTS ON KETOCAROTENOID SYNTHESIS IN *CHODATODESMUS AUSTRALIS*

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During a scientific expedition in Antarctica, in the austral summer 1990, Prof. C. Andreoli collected a coccoid microalga from a transitory pond near the Gondwana Station, Terra Nova Bay, Victoria Land (Antarctica). This organism was recently characterized by a polyphasic approach and resulted to be a new species of Chlorophyceae, named *Chodatodesmus australis* and belonging to the new genus *Chodatodesmus* (family Scenedesmeceae) (Sciuto et al., submitted). Physiological analysis showed the ability of this Antarctic isolate to grow at different temperatures (from 4°C to 25°C), with an optimum growth rate at about 20°C. *Chodatodesmus* resulted always able to synthesize antioxidant carotenoids as part of a photoprotective mechanism activated to avoid the risk of photooxidative damage by excess of light. In the present study, we tested the effects of different light regimes and nitrogen availability on biomass and carotenoid production in *C. australis*. For this purpose, we exploited a small photobioreactor (multicultivator MC1000, Photon System Instruments) with eight culture slots (80 ml), individually illuminated by a set of led light adjustable from 10 to 900  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ . Each slot is also equipped by an optical density detector, which measures every hour the turbidity at 750 nm, simplifying the evaluation of microalga growth curve. In all the considered conditions, morphology, lipid accumulation, chlorophyll and carotenoid content and photosynthetic activity, have been determined and correlated with the growth rate and biomass production. We also verified the nature of synthesized carotenoids by mass spectrometry analyses. They resulted to be mainly canthaxanthin, astaxanthin, ketolutein and their esters. Parallel to the ketocarotenoids accumulation, there was also a large production of lipids.

## 9OR.8

### SUCCESSION OF ALGAL AND CYANOBACTERIAL COMMUNITIES AFTER GLACIER RETREATS IN ALPINE - HIGH ARCTIC CLIMATIC ZONES IN NORTHERN EUROPE

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Accelerated melting of glaciers in many arctic and alpine areas due to global warming provides new habitats for biota colonization and soil formation and

thus a platform for primary succession and ecosystem development studies. Soil algae and cyanobacteria are usually first photoautotrophic organisms colonizing newly exposed terrain where they have to cope with extreme environmental conditions. To understand the role of particular groups/species of soil algae and cyanobacteria as well as main factors affecting development of microalgal communities during succession we selected forelands differing in climatic conditions, bedrock, altitude, slope, exposure, etc. The study was performed in foreland of 5 glaciers- Storglaciären (Tarfala, Sweden), Hardangerjokulen-Middalsbreen snout (Finse, Norway) and 3 glaciers in Petuniabukta (Svalbard) - along chronosequences established from recently deglaciated sites (early successional stage) at the glacier front to old successional stage developed since the end of LIA (climax). Soil samples were collected aseptically from surface layers. Species composition, dominant species/groups and abundance of algae were studied by using direct epifluorescence microscopy and cultivation methods; molecular methods were applied for identification of problematic species. Similar successional trend in algal community development was observed in all studied forelands. Six to 14 algal species were isolated already in the youngest, completely barren successional stages, small diatoms and xanthophytes (*Xanthonema*, *Heterococcus*) usually prevailed. Both species numbers and abundance rapidly increased with increasing successional age reaching more than 30 species and abundances typical for temperate soils, but in the oldest stages showed decreasing tendency. Cyanobacteria dominated usually in younger successional stages, and their occurrence decreased with decreasing soil pH, typically in old successional stages. *Pseudococomyxa*, *Elliptochloris*, *Chloroidium*, *Xanthonema*, *Leptolyngbya* spp. belonged to both most frequent and often dominant species. The study was supported by EU Programme [FP7/2007-2013] under grant agreement No 262693 [INTERACT] and project LD13046 [ME Czech Republic].

## 9OR.9

### ENDOLITHIC CYANOBACTERIA COMMUNITIES WITHIN EPISODICALLY DRY WATERFALL TUSA IN A SOUTHERN-CENTRAL ALPINE DRY VALLEY (VINSCHGAU, EUROPEAN ALPS)

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Aside of studies of “Tintenstriche” (= dark pigmented epilithic phototrophic communities on rock cliffs), records of endolithic Cyanobacteria from the European Alps are rare. We found a microbial community inhabiting an episodically dry-fallen surface of a waterfall tufa in the Alps, forming one or two distinct black to dark blue-green permanent endolithic layers in 1-2 mm and 4-6 mm below surface, and at a distance of 1-4 m from water-run areas. The waterfall tufa forms from ambient (non-thermal) waters during episodes of increased runoff. Community structure showing a prevalence of Cyanobacteria was studied by culture-independent and culture-dependent approaches and covered light- and electron microscopy (TEM) and molecular characterization using 16S rRNA-based clone libraries. Clonal inserts were screened by restriction fragment length polymorphism and individual restriction types were sequenced. The limestone was studied in epoxy-impregnated, petrographic thin sections under polarized light- and dark-field microscopy. Although there is a relationship of the endolithic community to the surface-dwelling “Tintenstrich” microflora, we found a smaller number of taxa within the endolithic layers, which seem specifically adapted to this microenvironment. The endolithic microbial communities presumably consist mainly of limestone-dissolving taxa rather than bio-calcifying taxa. The sub-millimeter sized pore spaces (parallel underneath the outer calcified surface of the waterfall tufa) they inhabit is filled with damp air rather than water; this enables the community to withstand extended periods of drought as typical of central-alpine valleys during summer. The functional groups of Cyanobacteria in the endolithon are composed of, both, nitrogen fixers (*Dichothrix* sp.) and non-N-fixers (*Aphanothece* sp.), adapted to low-light conditions; in the epilithon high-light and desiccation-tolerant taxa were recorded (*Gloeocapsa alpina*, *Gloeocapsopsis* sp., *Chondrocystis* sp.). *Nostoc* sp., however, was common in the cultures from both habitats.

#### 9OR.10

### **BIODIVERSITY OF STRESS TOLERANT CYANOBACTERIA ON EXPOSED SURFACES OF MONUMENTS OF CULTURAL HERITAGE, THEIR BIODETERIOGENIC ACTIVITY AND POSSIBLE CONTROL FOR CONSERVATION**

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Cyanobacteria are oxygenic phototrophic prokaryotes with a remarkable range of metabolic capabilities, morphologies and life styles. Hence, we are studying to understand the gene structures and functions of these organisms from several stressed habitats of India with an aim to explore their potential for using in biotechnology. The culture collection at our lab has a deposit of >200 species/strains of Cyanobacteria and many of these have been isolated from several stressed habitats like exterior of stone monuments, building facades and biofilms on sun-scorched soils. All these taxa have been identified following morphometric protocols, most validated following molecular protocols, e.g. 16 S rRNA sequencing and further work is currently ongoing at our group to develop an atlas of such microbes in a global scenario. Studies are also focused to understand the structural and molecular basis of the organism's performance under a set of environmental conditions by employing few selected nitrogen fixing Cyanobacteria species under *Tolypothrix* and *Scytonema*, those survive in desiccated state as biofilms on dried soils and exposed surfaces of building stones in India. In this regard using oligonucleotide probes deduced from a conserved region of amino acids of SOD (superoxide dismutase) proteins, a SOD gene was detected in *Hassellia byssoidea* (formerly *Tolypothrix*). Amplification of specific genes of the genomic DNA and sequencing of the PCR product yielded a *sodB* gene of 292 bp size. Southern hybridization confirmed the presence of a single *sodB* gene in the desiccation tolerant cyanobacterium. Another reason for effective colonization of these organisms on exposed stones even under hot summer of the tropics due to presence of UV-A and B absorbing pigments in their cells and their sunscreen role was characterized. Control of epilithic biofilms for arresting biodeterioration and conservation of monuments was studied, however, development of a cost effective method is still in process.

#### 9OR.11

### **RESPONSE OF BENTHIC DIATOMS INHABITING INTERTIDAL FLATS TO ENVIRONMENTAL STRESSES**

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Intertidal flats belong to the most productive ecosystems, largely owing to the primary production of diverse communities of benthic diatoms. They can maintain high levels of photosynthetic productivity despite the often harsh environmental conditions that



can potentially generate intracellular oxidative stress. The amplitude and combinations of stresses partly depend on the flat sediment type (typically cohesive fine particles *versus* less cohesive larger particles), that are inhabited by different diatom growth forms. Two main growth forms can be distinguished: motile epipelon, which inhabits fine cohesive sediment, and smaller, immotile or motile epipsammon which lives attached to particles in less cohesive sediments. In order to protect their photosynthetic efficiency, benthic diatoms have evolved behavioural and physiological processes. Behavioural processes mainly rely on the ability of some species to vertically move in the sediment in order to escape stressful conditions. Among the physiological processes, the non-photochemical quenching (NPQ), and the associated xanthophyll conversion (XC) and LHCx protein synthesis, allow the fast regulation and the safely balance of photosynthesis. We have examined the ability of representatives of the main growth forms of benthic diatoms as well as the one of natural communities to respond to simulated Spring and Summer potentially stressful conditions of light, temperature and salinity in combination or not. The results showed that i) the physiological response of benthic diatoms to environmental stresses is mainly related to their habitat and growth form; ii) the high light and high temperature are major stresses compared to high salinity. Our study also highlights the joint role of environmental harsh conditions in limiting transiently (i.e. during emersion), the photosynthetic productivity of benthic diatoms *in situ*.

## 9OR.12

### MINIMIZING EXCESS LIGHT ABSORPTION BY BENTHIC DIATOMS: COMPARATIVE KINETICS OF VERTICAL MIGRATION AND NON-PHOTOCHEMICAL QUENCHING

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Microalgae inhabiting intertidal flats are routinely exposed to high levels of solar radiation for prolonged periods. During low tide, the exposure to direct sunlight combined with extreme temperatures, high salinities and desiccation conditions is prone to cause photoinhibitory damage. Estuarine microphytobenthos (MPB) is typically dominated by pennate benthic diatom species that rely on a diadinoxanthin-xanthophyll cycle as the main

photoprotective process against photoinhibition. These diatoms use vertical migration to regulate light exposure and minimize light stress, a mechanism shown to represent an effective form of behavioural photoprotection. This study compares the short-term kinetics of the activation of both processes when cells are faced with potentially damaging light levels. Induction kinetics of the xanthophyll cycle was quantified on suspensions of MPB diatoms, by measuring the activation of non-photochemical quenching (NPQ) upon exposure to high light ( $1350 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) using a Pulse Amplitude Modulated (PAM) fluorometer. Vertical migration was quantified by measuring the short-term change in surface microalgal biomass on undisturbed sediment samples, using the optical index NDVI (Normalized Difference Vegetation Index). NDVI was measured on samples simultaneously exposed to a range of incident irradiance levels ( $50\text{-}1350 \mu\text{mol m}^{-2} \text{s}^{-1}$ ), generating Biomass-Light Curves. This was achieved by using a modified chlorophyll fluorescence imaging system with red and infrared bandpass filters, in combination with the projection of digitally-controlled, spatially separated light beams. The results showed that high light-induced vertical migration occurs at a much faster rate than previously reported, with surface biomass decreasing by more than 35% of total induced change during the first 2.5 min of light exposure. The comparison of the induction kinetics of NPQ and vertical migration showed that they are activated at comparable time scales, indicating a complex interdependency in the regulation and operation of the two photoprotection mechanisms.

## 9OR.13

### FIFTY YEARS AFTER POWELL: THE DISTRIBUTION OF *FUCUS DISTICHUS ANCEPS* ON THE EXTREME EXPOSED ROCKY SHORES OF ORKNEY AND ITS ROLE AS AN INDICATOR SPECIES

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*Fucus distichus anceps* is a boreal brown alga typically found on exposed mid-littoral rocky shores. The southern-most distribution extends to the north and west of Scotland and Ireland in often isolated populations associated with extreme wave energy. In 1957, H. Powell published two important papers on the ecology and distribution of *F. distichus* and updated this in 1963 with a more complete description of the distribution of *F. distichus anceps* in Orkney and

Shetland. Since then, only a limited number of macroalgal studies have featured this seaweed owing to its limited geographical range in British waters and the challenges in accessing much of its habitat. Interest however has returned following the identification of *F. distichus anceps* as a potential indicator species for changes in both climate (most obviously manifested as increases in sea surface temperature) and wave energy (targeted for extraction by wave energy converting devices [WECs]). We have established a long-term monitoring programme for *F. distichus anceps* while researching the role of wave energy on biological community dynamics on the rocky shores of Orkney – an area where WECs are being tested for large-scale deployment. Our research has included many previously unaccessed sites. All the locations described by Powell have been revisited and evaluated; abundance measurements of about 40 rocky shore species (including additional fucoids and important grazers) have been recorded; and various topographical features associated with littoral wave exposure have been measured. Topographic variables include: slope, complexity, aspect and orientation to fetch. Our research, in combination with Powell's records, suggests that the presence/absence of *F. distichus anceps* at individual site locations is remarkably stable on a temporal scale of decades. On Orkney, abundance of *F. distichus anceps* appears primarily to vary in relation to wave energy. We believe that this species is an important indicator of changes in wave energy but that its role as a climate change indicator has not yet been demonstrated.

#### 9OR.14

##### COMPARATIVE ANALYSIS OF MARINE AND FRESHWATER BROWN ALGAE: INSIGHTS INTO THE BIOLOGY AND EVOLUTION OF AN EXTREMOPHILE *ECTOCARPUS*

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*Ectocarpus* is a cosmopolitan brown algal genus with high capacity to acclimate to different environments. Particularly, wide tolerance ranges were observed with respect to salinity: while most *Ectocarpus* isolates are marine, at least one strain (named hereafter FWS) was isolated in 1996 from a true freshwater habitat at Hopkins River Falls in Australia. Indeed, the transition from marine environments to freshwater is a particularly rare event in brown algae, and only about 1% of brown algal species have colonized this habitat. Additional *Ectocarpus* isolates closely related to FWS have been found in other “extreme” environments such as on driftwood, or in areas of highly variable temperature, and belonged to the clade 2d of the *Ectocarpus* genus. Very recently, it has been proposed to reinstate the species *E. subulatus* for individuals referred to this clade, which is distinct, but closely related, to the species corresponding to the genome-sequenced strictly marine *Ectocarpus* (clade 1c), both having diverged roughly 16 Mya. The *E. subulatus* FWS is able to grow in both seawater and freshwater, showing that the transition towards the latter is not irreversible yet. This euryhaline strain exhibited profound, but reversible, morphological, physiological, and transcriptomic changes when transferred between freshwater and seawater, e.g. adjusting its metabolism and cell wall structure to the corresponding conditions. To elucidate the evolution of the FWS and its phenotypic plasticity, the genome of this strain (diploid) and of one of its close relatives (haploid) have been sequenced, and comparative analysis with this of the marine *Ectocarpus* is underway. Finally, we have shown recently that the ability of the FWS to grow in freshwater is strictly dependant of its associated phycosphere. Therefore, the FWS and its related microflora form an interesting biological system useful to assess how aquatic photosynthetic holobionts behave to face changes in environmental conditions.

#### 9OR.15

##### HOW CAN *ULVA INTESTINALIS* RESIST THE SUNLIGHT WHILE FLOATING ON THE SEA SURFACE?

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The green macroalga *Ulva intestinalis* is a key species of the upper intertidal zone often forming dense stands. The morphology with unbranched, partially gas filled tubes floating on the water surface renders it a true high-light plant which is exposed to unattenuated sunlight not only during low tide. Despite this regular sunlight exposure *U. intestinalis* contains no protective ultraviolet-B (UVB, 280 – 315 nm) screening pigments. It was examined how *U. intestinalis* copes with the high intensity of photosynthetically active radiation (PAR) and with the concomitant high dose of UVB of sunlight on several daily courses *in situ* and a long-term study in mesocosms. Our results show a remarkable potential of down regulation of photosystem II (PS II) efficiency indicated by a substantial quench of ground fluorescence ( $F_0$ ). Moreover, the recovery kinetics of PS II efficiency during the afternoons showed no delay implying a fast reversible photoinhibition. In addition, the regeneration of photodamaged PS II seems to be very efficient in *U. intestinalis*. Inhibitor studies after a UVB challenge revealed a very high capacity of PS II recovery by protein synthesis in this alga, being 4 times higher than in three other green macroalgae from the eulitoral. In summary, protective down regulation and repair processes act together to render the photosynthesis of *U. intestinalis* resistant against high PAR and UVB intensities. Surprisingly, no accumulation of UVB-induced DNA damage was detectable in the field although the solar irradiation was maximal for Northern Germany. From preliminary results we hypothesize that the photoreactivation of DNA dimers plays a crucial role for the DNA stability of *U. intestinalis* under solar irradiation.

#### 9OR.16

##### THE PHYSIOLOGICAL RESPONSES OF GIANT KELP *MACROCYSTIS PYRIFERA* TO OCEAN ACIDIFICATION ARE MODULATED BY INTERNAL NITROGEN STATUS

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The combined effects of elevated CO<sub>2</sub> (aq) and nitrate (NO<sub>3</sub><sup>-</sup>) supply on the carbon and the nitrogen physiology of *Macrocystis pyrifera* (hereafter *Macrocystis*)

were investigated. We hypothesized that: (1) NO<sub>3</sub><sup>-</sup> assimilation, the size of the internal NO<sub>3</sub><sup>-</sup> pool, and total tissue N content, will depend on NO<sub>3</sub><sup>-</sup> supply and (2) a higher CO<sub>2</sub> concentration will increase photosynthesis and growth of *Macrocystis*, coupled with an increase in NO<sub>3</sub><sup>-</sup> uptake and assimilation. *Macrocystis* discs were first grown under low (5 μM) and high (80 μM) NO<sub>3</sub><sup>-</sup> concentrations so that internal NO<sub>3</sub><sup>-</sup> pools were either nitrate deplete or replete. The discs were subsequently grown under current (400 μatm; pH 8.05) and future (1200 μatm; pH 7.59) CO<sub>2</sub> concentrations, with 20 μM NO<sub>3</sub><sup>-</sup>. After 3 days, the total tissue N content, nitrate reductase (NR) activity, NO<sub>3</sub><sup>-</sup> uptake, and internal NO<sub>3</sub><sup>-</sup> pool were completely modulated by the NO<sub>3</sub><sup>-</sup> concentration in seawater. NR activity, internal NO<sub>3</sub><sup>-</sup> pool and total tissue N content were reduced for discs initially grown under low [NO<sub>3</sub><sup>-</sup>]. An additional 3 day incubation in the CO<sub>2</sub> treatments had no effect on photosynthetic rates, growth rate, and NR activity, irrespective of the size of the internal NO<sub>3</sub><sup>-</sup> pool. However, higher NO<sub>3</sub><sup>-</sup> uptake and internal NO<sub>3</sub><sup>-</sup> pools were observed under elevated CO<sub>2</sub>, but only in discs with an depleted internal NO<sub>3</sub><sup>-</sup> pool. Results show that increased CO<sub>2</sub> did not enhance either photosynthesis or growth of *Macrocystis*, and NO<sub>3</sub><sup>-</sup> assimilation was mainly regulated by NO<sub>3</sub><sup>-</sup> availability. However, the higher NO<sub>3</sub><sup>-</sup> uptake observed in N-deplete blades indicates that N status may modulate the physiological responses of *Macrocystis* to OA.

#### 9OR.17

##### THE ALGA THAT NEVER READ THE LITERATURE - FASTEST GROWING, DESICCATION AND PHOTODAMAGE TOLERANT ALGA, ISOLATED FROM DESERT CRUST

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After many years of research, some feel that we have elucidated the functioning of the photosynthetic machinery and limitations therein, and what sets the upper limit for algal growth. However, the unparalleled fast growth, extremely high photosynthetic rates and resistance to photodamage, salinity and desiccation displayed by *C. ohadii*, recently isolated from a biological desert sand crust (BSC), one of the harshest environments to support life, suggest this may not be the case. When grown under optimal laboratory or controlled outdoor facility conditions it exhibits the fastest growth rates ever reported for an alga. The cultures perform among the highest photosynthetic rates ever reported and reach very high cell densities

( $1.3 \times 10^9$  cells/mL). When grown in photo-bioreactors under optimal conditions, division times as short as 1.2 h were recorded. To the best of our knowledge it is the fastest growth rate ever reported for a photosynthetic organism and close to the maximal attained by unicellular eukaryotes. Unlike other photosynthetic organisms, *C. ohadii* productivity is unaffected by irradiances as high as twice full sun light; and the level of protein D1, encoded by a single gene in the *C. ohadii* genome (56 MB), is hardly affected. Following exposure to high irradiance, *C. ohadii* cells undergo major structural (i.e. pyrenoid development and increased thylakoid abundance) and compositional changes (i.e. a 2-3 fold increase of the lipid and carbohydrate contents). In view of the unparalleled growth and photosynthetic performance, *C. ohadii* may be used in future research to clarify the processes that rate-limit growth and productivity of photosynthetic organisms. In an era where novel approaches to raise biomass production for food or energy supply are explored, *C. ohadii* may be used either directly in algal farms or as a gene source for the development of crop plants that can maintain high yields in environments not suited to current cultivars.

#### 9OR.18

##### AUTOTROPHS IN ANTARCTIC MELT-WATER MICROBIAL MATS - THE TOUGH SURVIVORS

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Autotrophs inhabiting polar meltwater microbial mats are exposed to extreme environmental conditions. These extreme conditions include large fluctuations in ambient radiation (including ultraviolet radiation), temperature, salinity and desiccation. We studied microbial mats growing in meltwater streams (water depth varying between 0.5 and 2 cm) close to Potter Cove, King George Island, January to February 2015. The dominating autotrophs were pennate diatoms and cyanobacteria. In four different experiments, the effects of ultraviolet radiation, temperature (freezing), elevated salinity and desiccation were tested in laboratory and outdoor set-ups. Measured variables differed between experiments and included photosynthetic activity (pulse amplitude modulated fluorometry, PAM), non-photochemical quenching (PAM), rapid light curves (PAM), species/genera identification, biovolume and growth (microscopy), photosynthetic pigments (HPLC), lipid peroxidation (thiobarbituric acid reactive substances (TBARS) assay) and biomass of heterotrophic bacteria. Samples are currently

transported to Sweden for chemical analyses. Over three weeks in ambient radiation, preliminary results from PAM measurements and cell numbers show that the microbial community were tolerant to both ultraviolet-A and ultraviolet-B radiation (reflected in total cell numbers and photosynthetic activity, Fv/Fm). Photosynthetic pigment and species/genera composition remains to be analysed. Furthermore, rapidly increasing salinity from freshwater to salinity 30 did not have any effect on photosynthetic activity (Fv/Fm) over nine days. This suggests that parts of the meltwater microbial mats may survive when flushed out in seawater and could contribute to primary production in Potter Cove. In conclusion, our preliminary results confirm that these microbial mats show a remarkable tolerance to extreme environmental conditions and are most likely survivors also in a rapidly changing climate at South Shetland Islands.

#### 9OR.19

##### EXPRESSION OF GENETIC TRANSPOSABLE ELEMENTS UNDER THERMAL STRESS IN THE DIATOM *PHAEODACTYLUM TRICORNUTUM*

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A significant part of virtually all genomes is made of genetic transposable elements (TEs) that are segments of DNA encoding enzymes that mediate their move within genomes. Most of them are inactive because of accumulated mutations but some of them are potentially active and may be mobilized, particularly in stressful conditions. Such mobilization is often harmful for the individual but promotes adaptability for the species since the mobility of those elements brings genetic diversity and largely contributes to genome evolution. Retrotransposons constitute a category of TEs that are particularly reactive to stressful conditions. Phytoplankton is subjected to variation in temperature that will keep increasing because of climate change. We analyzed the transcriptomic expression, in condition of thermal stress, of two retrotransposons previously characterized in the diatom *Phaeodactylum tricorutum*: *Blackbeard* and *Surcouf*. Whereas the expression of *Blackbeard* was not affected by thermal stress, *Surcouf* was overexpressed at high temperature, just as *small Heat Shock Proteins*



(*sHSP*). We searched Heat Shock Response motifs in the *sHSP* promoters and in the 5' DNA sequences - that are equivalent to promoters - of both retrotransposons. Heat Shock Elements (HSEs), Stress Response Elements (STREs) and/or CCAAT boxes were identified in the sequences of *sHSP* promoters and in the 5' DNA sequence of *Surcouf*, but not in that of *Blackbeard*. This result suggests that *sHSP* and *Surcouf* might be partly co-regulated and explains the overexpression of *Surcouf* at high temperature.

## 9OR.20

### A NEW GREEN ALGAL LINEAGE ISOLATED FROM A VOLCANIC CANYON IN THE CANARY ISLANDS

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Algae are one of the most diverse and highly adapted forms of life on Earth. Here, we report the discovery of a terrestrial green alga isolated from a peculiar habitat in La Palma (Canary Islands, Spain) – a steep volcanic canyon that is constantly exposed to changing weather condi-

tions (floods versus droughts). To understand how the alga adapted to survive and reproduce in this environment, we used axenic cultures, simulated the above-listed stresses and studied their effect on the alga's life history. We found that despite its relatively simple morphology (i.e. a uniseriate filament with intercalary cell divisions), the alga adapted to the fluctuating environment of the canyon through specific life-history traits. (1) Aplanospores enable survival during long drought periods, spreading during floods, and subsequent rapid (re)colonization due to abnormal first cell divisions in germlings leading to the formation of two or even several filaments from one aplanospore. (2) Fragmentation through 'filament splitting' (a unique mechanism, not reported before in green algae and initiated by formation of oblique cross walls) that takes place when the canyon begins to dry out (branch formation) and floods/heavy rain showers occur (fragmentation). (3) Besides that, the alga reproduces asexually during an aquatic life stage by zoospores (numbers of flagella per zoospore differ from 4-24) and may spread and thus maximize colonization during post-flood periods when submerged. Since the observed life history traits could not be assigned to a described taxon, we examined the phylogenetic position of this organism. Interestingly, we found that the alga represents a novel lineage at the genus and family level in the Chaetophorales (Chlorophyceae). In conclusion, our findings clearly indicate that the study of 'non-typical' habitats can help to discover novel microalgal diversity and provide new insights into evolutionary processes that have shaped adaptations of such algae to their specific *habitat*.

## 10. Global change and algal assemblages: the fate of our seas

### 10KN.1

#### VANISHING FORESTS OF CANOPY ALGAE: A GLOBAL PROBLEM WITH LOCAL SOLUTIONS?

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Canopy-forming seaweeds are severely retracting globally, particularly in urban areas, and are replaced by turf-forming algae or sea urchin barrens. Those forests that remain are under continued and underestimated threat, and the overall benefits of current protection measures have been low. I analyse the drivers of loss of these highly threatened and valuable ecosystems. These involve complex interactions between multiple local (nutrient enrichment and high sediment loads, fishing, heavy metal pollution) and global stressors (increasing temperature, high wave exposure, and high UV or CO<sub>2</sub>). While many of the effects of global threats will continue for decades, if not centuries, experiments suggest that the control of local stressors could work as a rapid and cost-effective strategy to limit at least some of the adverse effects, allowing more time to these ecosystem to adjust to climate change. A resilience approach to limit the impacts of global climate changes by managing synergies between global and local stressors is illustrated with reference to the experience developed within project TETRIS (Observing, modelling and Testing synergies and TRade-offs for the adaptive management of multiple Impacts in coastal Systems).

### 10KN.2

#### ECO-EVOLUTIONARY DYNAMICS IN PLANKTON COMMUNITIES

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Coevolutionary changes in antagonistic interacting populations can constantly change the mode, strength and direction of selection through the link between the ecological and evolutionary dynamics (eco-evolutionary

dynamics). To explore the role of eco-evolutionary dynamics and their effects on selection over time, we conducted a series of experimental evolution studies using an algal host and its virus. We will present results from experiments where we compared the evolutionary and ecological dynamics of experiments when host and virus coevolved with and without an additional predator in the system. We found that predator and virus could only coexist after the host evolved a general resistance against the virus and that coexistence of the two consumers shifted the ecological dynamics towards cyclic populations compared to the experiments with only host and virus. Most importantly, the presence of the predator changed the evolutionary dynamics from fluctuating selection when host and virus evolve alone, to constant selection when virus and predator coexisted. Overall, our study shows the important consequences of rapid evolutionary change and highlights that the details of coevolutionary dynamics matter for community stability (cycles vs. stability) and structure (coexistence or not), which in return determines type of selection (fluctuating vs. constant).

### 10OR.1

#### INVESTIGATING TEMPORAL CHANGES IN SEAWEED COMMUNITIES OF BRITTANY

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Brittany is a hotspot of macro algal biodiversity located at the confluence of the Lusitanian and the boreal ecoregion. Population genetic studies have shown that it was a refugium for macro algae during the Last Glacial Maximum. Our study of evolution of coastal water temperature in Brittany showed that the temperature increased by 0.7 °C on average over the past two decades (0.35 °C per decade). At a finer scale, changes in SST showed that Brittany constitutes a mosaic of contrasting conditions, with the western and north-western regions being colder and less affected by climate change than the other three regions. We analysed changes in macro algal communities by i) assessing turn over of subtidal red seaweed assemblages over the 20-year period and ii) modelling distribution changes for selected species with species distribution models, iii) evaluating from natural history collection accommodated in marine stations the sweep and the tempo of those changes over 200 years.

## 10OR.2

### NE ATLANTIC *CORALLINA* (RHODOPHYTA) IN A HIGH CO<sub>2</sub> WORLD

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A comprehensive study of the phylogenetics and ecophysiology of species of the calcified red algal genus *Corallina* was undertaken across the NE Atlantic to provide projections of the fate of these ecosystem engineers under a high CO<sub>2</sub> world. *Corallina* species are ubiquitous in temperate intertidal habitats, although they are predicted to be particularly vulnerable to the impacts of ocean acidification and ocean warming. The establishment of clear species concepts for *Corallina* present in the NE Atlantic facilitated a subsequent phylogenetic study that explored species diversity, distributions and the extent to which names have been misapplied. From this baseline, investigations into the ecophysiology (productivity, calcification, growth and skeletal mineralogy) of *C. officinalis* and *C. caespitosa* were performed across the NE Atlantic (Iceland to northern Spain) in relation to temporal and spatial fluctuations and gradients in temperature, irradiance and carbonate chemistry. Results

highlighted significant cryptic diversity within the genus and demonstrated the cosmopolitan distribution of *C. caespitosa* in contrast to the restricted North Atlantic distribution of *C. officinalis*. *Corallina* species were shown to experience significant seasonal and tidal fluctuations in abiotic conditions, which may infer resilience to future change in seawater acidity, carbonate saturation and water temperature. Productivity, calcification and growth followed seasonal cycles in abiotic parameters, although decreasing growth was observed with increasing latitude. Seasonal cycles in skeletal Mg incorporation were apparent, with a strong positive relationship to sea surface temperature, although no significant change in skeletal mineralogy was evident in herbaria collections spanning from pre-industrial times to the present. Overall, these findings indicate that *Corallina* species have the potential to survive under future acidification and ocean warming conditions, although loss of populations from higher latitudes and shifts in the relative abundance of species across lower latitudes of the NE Atlantic are projected.

## 10OR.3

### THE WEAKEST LINK: ARE THE MICROSCOPIC STAGES OF SEaweEDS MOST SUSCEPTIBLE TO CLIMATE CHANGE?

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Seaweeds are foundation species that provide 3-dimensional habitat and food for invertebrates and fish. All seaweeds have complex life cycles, with unicellular and microscopic cryptic stages that may be more vulnerable to climate change than their corresponding adult stages upon which most research has focussed. There has been virtually no comprehensive research on the interactive effects of different climate change stressors on the survivorship of these microscopic stages of seaweeds, nor studies on physiological or biochemical mechanisms that might confer an ability to acclimate to climate change. This lack of research on microstages is surprising given that they are the seed bank for the next generation of the ecosystem-engineering macroscopic population, and that change in the viability of microscopic stages and decrease in their recruitment success will have profound flow-on effects to the macroscopic community and the ecosystem they support. Knowledge on species' survival thresholds is key in preserving marine biodiversity.

**10OR.4****INSIGHTS FROM *OSTREOCOCCUS* INTO COPING IN A SEA OF VIRUSES AND COMPETITORS**Sheree Yau ([sheree.yau@obs-banyuls.fr](mailto:sheree.yau@obs-banyuls.fr))*Integrative Biology of Marine Organisms, Banyuls Oceanologic Observatory, Banyuls-sur-Mer 66650, France*

The smallest free-living eukaryotes are picoalgae of the genus *Ostreococcus*, which measure less than 1 μm diameter. Four clades based on 18S ribosomal gene phylogeny (A-D) have been defined based on >98% nucleotide identity that correspond with coastal, surface ocean and deep ocean ecotypes. The genetic distance between these clades in the rest of the genome is enormous with orthologous genes sharing 72% identity [3]. Molecular phylogeny and karyotyping indicates the 18S-defined clades represent distinct species, and not simply ecotypes. Despite this large genetic diversity, *Ostreococcus* species are morphologically indistinguishable. The type species, *O. tauri* (clade C) and the newly described species, *O. mediterraneus* (clade D) appear to both be most abundant in coastal Mediterranean lagoons. However, phylogenomics indicates *O. mediterraneus* is the deepest branching lineage within *Ostreococcus*. Genome sequencing of *O. mediterraneus* was performed to determine the expanded gene families, unique genes in this species and gain insight into the evolution of the genus. From transcriptomes of several strains of *O. mediterraneus*, the nucleotide diversity estimated was consistent with levels in intraspecies populations. Positive tests for recombination indicate *O. mediterraneus* occurs as a distinct panmictically breeding species. Surprisingly, the type strain of *O. mediterraneus* chronically produces infective viruses but is not lysed. These data shed light into how sympatry has evolved in *Ostreococcus* and implicates a role for viruses.

**10OR.5****WARM & ACID: CHANGES ON SPECIES INTERACTIONS IN THE NEW OCEAN**Francisco Arenas<sup>1</sup> ([farenas@ciimar.up.pt](mailto:farenas@ciimar.up.pt)), Maria Jose Araujo<sup>1</sup> ([maria.jmar.araujo@gmail.com](mailto:maria.jmar.araujo@gmail.com)), Eduardo Sampaio<sup>1</sup> ([edusilvasampaio@gmail.com](mailto:edusilvasampaio@gmail.com)), David Calvao<sup>1</sup> ([davidcalvao@hotmail.com](mailto:davidcalvao@hotmail.com)), Ivan Rodil<sup>2</sup> ([ibantxorodil@gmail.com](mailto:ibantxorodil@gmail.com)) and Joao Franco<sup>1</sup> ([joaonunofranco@gmail.com](mailto:joaonunofranco@gmail.com))<sup>1</sup>*Aquatic Ecology & Evolution Group, CIIMAR, Porto 4050-123, Portugal and* <sup>2</sup>*Aquatic Ecology & Evolution Group, CIIMAR, CIIMAR 4050-123, Portugal*

Rising levels of atmospheric CO<sub>2</sub> and increasing temperatures altered ocean climate with well documented harmful effects on marine ecosystems. In the case of seaweeds, ocean warming and acidification have large effects not only on their metabolic rates and population dynamics, but also on the way they interact with other trophic levels like seaweed grazers. Given the importance of top-down effects on the regulation of seaweed assemblages, predicting the ecological impacts of ocean climate change requires not only studies on the ecophysiological effects but also research on species interactions. Here we present a set of studies aimed to understand how the combined effects of seawater warming and acidification shape seaweed assemblages and their herbivore interactions. We carried out a set of mesocosm experiments including crossed treatments of pH and temperature where we measured short term functional responses of different species of seaweeds and grazers to future environmental conditions and also changes on the intensity of macroalgae-herbivore interactions. Our results showed that species response to ocean climate change were non-linear and differed among species. Also synergistic interactions among physical drivers may hinder our ability to predict the future impacts of climate change.

**10OR.6****RECOVERY OF *CYSTOSEIRA* FORESTS ALONG THE WEST ISTRIAN COAST (NORTHERN ADRIATIC SEA, CROATIA)**Ljiljana Iveša ([ivesa@cim.irb.hr](mailto:ivesa@cim.irb.hr)) and Tamara Djakovac ([djakovac@cim.irb.hr](mailto:djakovac@cim.irb.hr))*Center for Marine Research, Ruđer Bošković Institute, Rovinj 52210, Croatia*

The west Istrian Coast delimits the eastern border of the northern Adriatic Sea. Because of increased levels of nutrients due to runoffs from the Italian rivers, the northern Adriatic is one of the most eutrophicated regions of the Mediterranean Sea. In total, eight *Cystoseira* species were historically recorded along the west Istrian Coast during the middle of the past century (*Cystoseira amentacea*, *C. barbata*, *C. compressa*, *C. corniculata*, *C. crinita*, *C. foeniculacea*, *C. humilis* and *C. spinosa*). Historical data show that approximately from 1975 to 1990 *Cystoseira* populations were in a phase of regression because of increased eutrophication levels and sea urchin grazing. A decreasing trend of river runoffs and the prohibition of phosphate-based detergents during 1980s produced an oligotrophication of the northern Adriatic. Concomitantly with the decrease in eutrophication levels and a decrease of sea urchin populations, *Cystoseira* spp. recovered so that monospecific



and mixed *Cystoseira* forests have recolonized the majority of rocky bottom habitats of the west Istrian Coast after year 2000. The recovery occurred in spite of the fact that nearly each year from 1989 to 2004 massive mucilage aggregations have formed in the water column of the northern Adriatic provoking mass mortality of macrozoobenthos species. It could be suggested that the decrease of sea urchin density was a consequence of the mucilage impact. On the contrary, northern Adriatic *Cystoseira* spp. seems to be adapted to cope with the mucilage phenomenon. In general, *Cystoseira* forests are in a phase of regression all over the Mediterranean Sea. The assessed recovery is likely related to the particular oceanographic and ecological characteristics of the northern Adriatic.

### 10OR.7

#### TEMPORAL BETA-DIVERSITY OF SMALL EUKARYOTE PHYTOPLANKTON IN A EUTROPHIZED TROPICAL COASTAL LAGOON

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Monitoring of coastal-ecosystem health requires indicators of community structure and function. The phytoplankton community index (PCI) considers the seasonal variation of the community within a multivariate space taking into account a reference envelope (with a ring-shaped envelope indicating a resilient ecosystem that quickly returns to its “natural” state after small perturbations). However, whether this concept could be applied in tropical habitats, with less marked seasonality, was never tested. Here, the temporal beta-diversity (i.e., the turnover of species over time) was used as a proxy of the PCI by using Non-metric Multidimensional Scaling (NMDS) coupled with analysis of similarities (ANOSIM), where R values closer to 1 denote a quick temporal species turnover. We combined microscopic analysis and 18S rRNA sequencing (cultures and clone libraries) to assess the phytoplankton temporal beta-diversity in Rodrigo de Freitas Lagoon (RFL), an important urban system subject to an intense anthropogenic impact at

the coast of Rio de Janeiro City, Southeast Brazil. We focused in the small eukaryote algae (<10 µm) as they are usually important components of phytoplankton communities in brackish coastal lagoons but seldom considered when assessing their environmental stability. Samples were collected twice a week from January 2012 to December 2013 at five sampling stations. A quick temporal turnover of species (few days) was observed as well as the absence of a stable and recurrent seasonality (global R = 0.8, p = 0.01). As a result, phytoplankton assemblages in the RFL did not return to their starting point after a year, but followed a path that can remove them far from their initial point. Temperature, salinity and NH<sub>4</sub> were the main abiotic variables explaining the observed temporal variability. High values of temporal beta diversity reported here are consistent with degraded habitats, subject to rapid environmental changes.

### 10OR.8

#### EFFECTS OF WARMING AND ACIDIFICATION ON A BENTHIC COMMUNITY IN THE BALTIC SEA – KIEL BENTHOCOSMS

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Changes in global temperature and ocean chemistry associated with increasing CO<sub>2</sub>-concentrations are the most wide-spread effects of climate change. Numerous studies have shown the impact of single stressors on individual species. However, the combined effect of multiple stressors on a multi-species assemblage, which are ecologically much more realistic and relevant, are still missing. Therefore, within the framework of the German BIOACID project, we orthogonally crossed the two factors warming and acidification in Benthocosm experiments and studied their single and combined impact on the keystone brown alga *Fucus vesiculosus* and its associated community in the Baltic Sea in all seasons (from April 2013 to April 2014). Importantly, to take into account the natural fluctuations of all environmental variables we superimposed our treatment factors onto these in the benthocosms. For that reason we worked with delta-treatments in all seasons, i.e. ambient Kiel Fjord temperature plus the predicted 5 °C of warming by the end of the century and the ambient pCO<sub>2</sub> plus the predicted change (+600 ppm) for the year 2100. In all seasons, temperature and, to a much lesser extent, CO<sub>2</sub> affected adult and juvenile *F. vesiculosus* physiology (growth, photosynthesis) including its associated epiphytes, biofilm and/or mesograzers. Late summer turned out to be the critical season for temperature impacts on the entire *Fucus* system. Epiphytes showed massive growth, which accelerated a die-off of *F. vesiculosus*. The epiphytic overgrowth could indirectly be explained by temperature-driven reduced top-down control, because the main mesograzers species declined under high temperature during summer as well. Understanding responses of macroalgae and of the associated community is important because changing global temperatures and elevated CO<sub>2</sub> may affect the ecological role of *F. vesiculosus* as primary producer, carbon sink, water purifier, and ecosystem engineer in the coastal ecosystem of the Baltic Sea.

## 100R.9

### RECENT PROGRESS IN THE PHYSIOLOGY, MINERALOGY AND ECOLOGY OF CORALLINE ALGAE IN THE GREAT BARRIER REEF AND IMPACTS OF FUTURE OCEAN CHANGE

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Crustose coralline algae (CCA) play important roles in tropical and temperate reefs. CCA contribute to coral reef growth and cementation and are pivotal for reef resilience by inducing coral larval settlement. However, CCA are sensitive to human-induced ocean acidification and warming. Given the importance of CCA for marine systems and the concerns about the impacts of projected oceanic change on CCA, we have developed a research program to address key knowledge gaps on the physiology, skeletal mineralogy and ecology of CCA in the Great Barrier Reef (GBR), Australia. In particular, our research focusses on improving our understanding of the effects of elevated pCO<sub>2</sub> and temperature on CCA and the mechanisms by which these effects occur. In this talk we will provide a summary of the research we have conducted on these topics in the last few years and will give an update on the projects we are currently doing in the GBR. I will present information on the physiological responses of early life stages of CCA to elevated pCO<sub>2</sub> and interaction with other factors. Further, I will discuss issues related to the variability in skeletal mineralogy among reef habitats and discuss how the mineralogical composition varies in response to changes in pCO<sub>2</sub> and temperature under experimental conditions. We have advanced our knowledge on how the process of calcification responds to changes in seawater carbonate chemistry and the existing inter-specific variability in the group. However, very little is known about the variability in net and gross calcification rates directly in the natural environment, and especially the interactions with water. Understanding the nature and magnitude of the responses of CCA to future ocean change will help us to assess whether coralline algae will continue to build and stabilise coral reefs as CO<sub>2</sub> rises, crucial information for environmental management agencies.

**10OR.10****ARE THERE ANY ALIEN MARINE DIATOMS?**David G Mann ([d.mann@rbge.org.uk](mailto:d.mann@rbge.org.uk))*Aquatic Ecosystems, Institute for Food and Agricultural Research and Technology (IRTA), Sant Carles de la Ràpita E-43540, Spain*

The last 30 years have witnessed a major shift in thinking about diatom biogeography, from an earlier perception that most species are widely distributed, at least within a broad climatic zone, to one in which species are assumed endemic to a region or island until proved otherwise. In part, this change is correlated with a shift to a narrower species concept, which was prompted by closer scrutiny of morphological variation, mating trials, and DNA sequence data. Moreover, microsatellite data indicate that populations of a single species are fragmentary on a regional scale, giving ample opportunities for allopatric and peripatric speciation. In the new ‘many endemics’ paradigm, it makes sense that particular diatom species might at times be transported outside their native range through human agency and disrupt the natural functioning of the communities to which they are introduced (just as has happened innumerable times with macroscopic animals, plants and seaweeds). There have been several claims that this has indeed occurred. Thus, for example, *Coscinodiscus wailesii* is supposed to have been introduced to European waters c. 1970 (perhaps with oyster spat or perhaps in ship ballast water) and to be ‘invasive’. The criteria for assessing such claims have not been clearly defined and will be discussed (e.g. good taxonomy consistently applied, appropriate search strategy, etc) with reference to selected planktonic and benthic diatom species. My conclusion is that there is no convincing evidence for introduction of any marine planktonic diatom (e.g. old collections show *C. wailesii* was already in the UK in the early 20th century). Conversely, application of the same criteria suggests that a benthic species, *Diademoides luxuriosa*, has been introduced to the UK from the Pacific. This single example indicates that introductions have probably been frequent and will likely increase, but in practice most will be undetected.

**10OR.11****EXPLORATION OF ARCTIC AND ANTARCTIC SEAWEED BIODIVERSITY IN THE CONTEXT OF POLAR CLIMATE CHANGE**Frithjof C Küpper ([fkuepper@abdn.ac.uk](mailto:fkuepper@abdn.ac.uk))*Oceanlab & School of Biological Sciences, University of Aberdeen, Newburgh AB41 6AA, United Kingdom*

Polar regions are hotspots of climate change, which inevitably also affects seaweed biodiversity by sea ice recession, increased iceberg scouring, and increased inputs of glacial melt water, all of which can have major impacts on phytobenthic communities. However, any studies of the polar phytobenthos in this context confront major challenges in terms of (1) scarce historic baseline datasets and (2) environmental and logistical constraints for scientific collections, and *in situ* observations by diving. This paper presents highlights from our expeditions to northern Baffin Island (Canadian Arctic, 2009) and Adelaide Island (Antarctica, 2010-2011) which assessed the biodiversity of seaweeds and associated eukaryotic pathogens at established study sites. Our dataset provides a baseline inventory for future work assessing impacts of the currently ongoing changes in the Arctic and Antarctic marine environment. In both cases, diving surveys and collections of macroscopic algae were complemented by applying the Germling Emergence Method and DNA barcode sequencing of the live isolates obtained from substratum samples. We present a baseline seaweed species checklist for northern Baffin Island in the Arctic and southern Adelaide Island/Northern Marguerite Bay in the Antarctic, respectively, reporting numerous new records of seaweed taxa and associated pathogens. The paper also discusses implications for establishing baseline inventories and managing safe and scientifically productive diving operations in remote polar locations.

**10OR.12****TEMPORAL AND SPATIAL POPULATION STRUCTURE AND GENETIC DIVERSITY DURING A BLOOM OF THE MARINE DIATOM *PSEUDO-NITZSCHIA MULTISTRATA***

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Addressing the question of the origin and maintenance of genetic diversity and structure in phytoplanktonic species is of fundamental importance to understand the population dynamics of these organisms, their capability to face and adapt to environmental changes and the possible mechanisms of speciation. We



assessed the population genetic structure of the toxic pennate diatom *Pseudo-nitzschia multistriata* in the Gulf of Naples (Tyrrhenian Sea) on a temporal and spatial scale, with the aim of clarifying the population dynamics behind patterns of genetic diversity. For the temporal survey, about 500 strains were isolated at the Long Term Ecological Research station MareChiara, with weekly sampling during the bloom period in summer-autumn 2013. For the spatial survey, about 200 strains were isolated from different locations along the Tyrrhenian coasts in July 2013. Strains were genotyped using 27 polymorphic microsatellite loci, of which 20 were designed on the *P. multistriata de novo* genome sequence. Two main blooms were recorded: one in August-September and a minor one in October. Genotypic diversity was high during the first bloom ( $G/N = 97.95$ ), but considerably dropped at the beginning of the second bloom ( $G/N = 24.61$ ), which was due to a few strains, one of them largely dominating the population. However, growth rates of the 'dominant' strain did not differ significantly from those of the other strains. Likely, other mechanisms of intra-specific competition should be invoked to explain this case of clonal selection (e.g. parasite selection or allelochemical interactions). The spatial survey revealed a very high genetic diversity, but weak population structure, suggesting that a single spatially extended population acts as a source for the inoculum of the bloom.

#### 10OR.13

##### IRON LIMITATION IN THE CYANOBACTERIA *SYNECHOCOCCUS* SP.: FROM GENE EXPRESSION TO PHYSIOLOGICAL RESPONSES

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In up to 40 % of the ocean the growth of phytoplankton and carbon dioxide fixation is limited by the lack of iron (Fe). This essential micronutrient is particularly important for photosynthetic organisms because of their high Fe requirements for the photosynthetic machinery. Cyanobacteria have developed several strategies to survive in Fe limited environments. In this study, we characterize the homeostasis of the marine cyanobacteria *Synechococcus* sp. PCC 7002 growing in steady-state under three levels of iron limitation. Cultures were performed in chemically characterized synthetic seawater amended with increasing concentrations of Fe (0.6, 2, 20 and 200

nM). Samples for Whole Transcriptome Shotgun Sequencing (WTSS), concentration of intracellular ATP, particulate organic carbon (POC) and nitrogen (PON), and pigments were collected. Besides, photo-physiological parameters were determined, using a Fast Repetition Rate Fluorometer (FRRF), in dark acclimated samples as well as under increasing levels of photosynthetic active radiation (PAR). Significant gradual decrease of the photosynthetic efficiency (photosynthetic yield, non-photochemical quenching, electron transfer rate, etc.), growth rate and ATP cell quota, was obtained for the different treatments. Several studies have suggested that the physiological mechanisms to survive under different levels of Fe limitation were different. We also identify a sequential response to severity of Fe limitation at transcriptomic level. At each condition of limitation one assortment of unique genes lead showed specific signatures to Fe limitation stress. The combination the physiological and transcriptomic responses will contribute to a more complete picture on iron-stress homeostasis in cyanobacteria. Consequently, also contribute to the better understanding of the ocean biogeochemistry and the biological carbon pump that regulates our climate.

#### 10OR.14

##### BIOCHEMICAL ADAPTATION OF THE INVASIVE *GRACILARIA VERMICULOPHYLLA* ALONG A GRADIENT OF TEMPERATURE, FROM PORTUGAL TO NORWAY

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Seaweeds represent one of the largest groups of marine aliens in Europe, and constitute between 20 and 29 % of all invasive marine species. Alien species have been shown to be particularly adaptive



through phenotypic changes. Moreover, to colonize their novel environment, invasive species have to adapt their defense against epiphytes and grazers, by e.g. production of chemical cues. Abiotic factors regulating primary production, such as latitudinal variation in temperature, can also influence the biochemical adaptation in alien seaweeds. These aspects have not been studied previously in *Gracilaria vermiculophylla*. This species was recently observed along the European coasts, and is able to modify the estuarine habitat it invades, all along the European Atlantic coasts. In this context, this study assessed latitudinal variation in macromolecules of the invasive red macroalga *Gracilaria*

*vermiculophylla* across its Atlantic distribution, from Portugal to Norway. For this purpose, *G. vermiculophylla* was collected at three sites in each of three countries (Portugal, France and Norway). The total lipid and fatty acid composition were measured by HP-TLC and gas chromatography and pigments were quantified using HPLC and spectrophotometry. Moreover, chemical footprinting analyses were obtained by HRMAS NMR. Our results are discussed in regard to the variability of molecules in *G. vermiculophylla* along the temperature gradient. This work was financed with the support of the EU FP7 ERA-NET Program, Seas-Era INVASIVES project nr. ANR-12-SEAS-0002

## 11. Genetic engineering in algae: novel molecular tools and novel model species.

### 11KN.1

#### DIATOMS AS MODEL SYSTEM FOR ALGAE WITH SECONDARY PLASTIDS

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In the recent years research on molecular aspects of microalgae showed a breath-taking development. While in the beginning, the green alga *Chlamydomonas reinhardtii* was the only model system that allowed genetic approaches to study cellular functionalities, other non-chlorophyte model systems are catching up. Here diatoms are especially noteworthy as they not only were the first non-green microalgae for which a useful genetic transformation system had been developed, they also are excellent model systems for a better understanding of cellular processes involved in secondary endosymbiosis, silica cell wall formation as well as the physiological reasons for the profound ecological importance of diatoms. Besides the availability of annotated genome sequences of a number of diatoms, several approaches regarding reverse genetics have been developed recently including RNAi and genome editing using TALEN approaches. This is extremely important because (i) diatoms are diplonts, thus random mutations in single alleles may not lead to phenotypes, and (ii) most diatoms do not show sexual reproduction in the lab, thus the availability of using sexual crosses to obtain homozygous mutants is very limited. In this presentation, the current status of molecular genetic approaches for diatoms will be described.

### 11KN.2

#### DEVELOPMENT OF FORWARD GENETIC AND GENOMIC APPROACHES TO IDENTIFY KEY REGULATORY GENES IN THE BROWN ALGAE

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The brown algae are considered to be the most developmentally complex group of organisms after animals and land plants. These seaweeds include, for example, some of the largest organisms on the planet, the giant kelps, which can reach lengths of more than 50 metres. Brown algae are therefore clearly of interest for understanding the mechanisms underlying developmental processes but, at present, very little is known about the molecular basis of development in these organisms. About ten years ago we proposed the filamentous brown alga *Ectocarpus* as a general model organism for the brown algae. *Ectocarpus* was selected specifically because of the potential of this alga for the application of genetic and genomic approaches. A broad range of resources have now been developed for this organism, including a complete, well-annotated genome sequence, extensive mRNA and small RNA transcriptomic data, a genetic map and other genetic tools. These resources are currently employed to identify and characterise key regulatory loci associated with several developmental processes including, for example, the control of life cycle progression and sex-determination. Here we will review some of the genetic and genomic approaches that are used to identify these loci including classical positional cloning approaches using the genetic map, NGS-based cloning methods such as the SHOREmap approach, genome resequencing and genome-wide comparative methods.

### 11KN.3

#### ALGAL CHLOROPLAST ENGINEERING: NEW TOOLS, TECHNOLOGIES AND APPLICATIONS

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The last few years have seen an ever-increasing interest in the exploitation of microalgae as

recombinant platforms for the synthesis of novel bio-products. These could be biofuel molecules, speciality enzymes, nutraceuticals or therapeutic proteins such as antibodies, hormones and vaccines. This exploitation requires the development of new genetic engineering technologies for species suited for intensive commercial cultivation in bioreactor systems. In particular, there is a need for routine methods for the genetic manipulation of the chloroplast genome (plastome) for two reasons: firstly, the chloroplast genetic system is well-suited to the targeted insertion into the genome and high-level expression of foreign genes, secondly, the organelle is the site of numerous biosynthetic pathways and therefore represents the obvious ‘chassis’ on which to bolt new metabolic pathways that divert the carbon fixed by photosynthesis into novel hydrocarbons, pigments, *etc.* I will present recent progress in my group on the development of advanced tools for the engineering of the *Chlamydomonas* plastome, and give examples of possible applications. Specifically, we have developed new expression vectors, a recipient host and an easy DNA delivery method that simplifies the creation of homo-plasmic transgenic lines without the use of antibiotic-resistance markers. We have developed a negative selectable marker that allows, *inter alia*, the investigation of nuclear *trans*-acting factors that regulate the mRNA stability/translation of endogenous chloroplast genes, and thus the expression of transgenes fused to the UTR elements from these genes. We also have developed a simple codon-reassignment method to address both the problem of unwanted transgene expression in *E. coli* when creating transformation constructs, and the need for transgene containment within the polyploid chloroplast. Current applications include the production of anti-bacterial proteins for several major bacterium pathogens, and the introduction of enzymes for the synthesis of novel terpenoids.

### 11OR.1

#### THE DEVELOPMENT OF GENETIC TOOLS FOR COCCOLITHOPHORES

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Coccolithophores are the most prolific producers of calcium carbonate in the marine environment

and thus have a profound effect on the global biogeochemical cycles. Many coccolithophores synthesise calcite scales in a specialised intracellular compartment called the coccolith vesicle, then secrete the finished coccolith onto the cell surface. Coccoliths are morphologically complex, and their architecture is genetically encoded. An understanding of the mechanisms underlying the fine control of calcite crystal shape in coccolithophores would have relevance for the design of advanced materials. Our knowledge of the molecular mechanisms of calcification in coccolithophores is extremely limited. To further our understanding of this process and to maximise the biotechnological potential of coccolithophores, the development of tools for their genetic manipulation is highly desirable. We have focused on the development of genetic transformation systems for the abundant, bloom forming species *Emiliania huxleyi* and the coastal species *Pleurochrysis carterae*. We will discuss the particular challenges of working with coccolithophores, from limited antibiotic susceptibility, to the complexities of their life cycles as well as the challenges of delivering DNA into the cell and achieving integration into the genome. We will report the progress we have made in developing selectable marker cassettes under the control of native regulatory sequences and in developing selection systems based on antibiotic resistance and on the complementation of thiamine auxotrophy. Finally, we will present our progress in the establishment of DNA delivery systems, and our pipeline for the analysis of putative transformants.

### 11OR.2

#### BIOTECHNOLOGICAL OPTIMIZATION OF LIGHT USE EFFICIENCY IN NANNOCHLOROPSIS CULTURES

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Microalgae have reemerged as potential next-generation feedstock for biofuels and they are considered very promising on the long term, thanks to their high productivity per area and ability to grow on marginal land without competing with food crops. Solar radiation provides the energy supporting algae growth and lipids production and the available radiation must be exploited with the highest possible efficiency to optimize productivity and make their cultivation on a large scale economically competitive. Investigation of the bases affecting light use efficiency is thus seminal to improve lipid/biomass productivity of algae industrial cultivation. One major point to be considered is that algae in

photobioreactor/ponds are growing in an artificial environment and experience substantially different conditions with respect to the ones where these organisms evolved. In order to optimize algae cultivation is thus seminal to understand how this artificial environment affects productivity. This problem was faced by analyzing the influence of key parameters like light dynamics, nutrients availability, CO<sub>2</sub> supply to algae productivity in batch/ continuous cultures, focusing on the seawater microalgae *Nannochloropsis gaditana*, a species com-

bining a fast growth rate with a high accumulation of lipids. A combination of genomic, transcriptomic and metabolomics analyses were also employed to describe in detail cells response to different conditions, with the aim of identifying limiting steps for biomass and lipids productivity in this alga. All information obtained is currently exploited to drive efforts for *Nannochloropsis* genetic modification. Mutants with altered regulation of photosynthesis has been isolated and showed improved productivity in lab scale photobioreactors.



## 12. Ecology, physiology and taxonomy of freshwater phytoplankton

### 12KN.1

#### PHYSICAL AND BIOLOGICAL DISPERSAL BARRIERS IN BLOOM-FORMING MICROALGAE

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Many freshwater and marine phytoplankton are widespread, and it is therefore assumed, that they disperse easily and frequently. However, many recent studies have shown both high genetic diversity and biogeographical patterns. My approach to address this issue is to investigate genetic diversity and dispersal at the population rather than species level. Specifically, I will present the results from a collaborative project focused on the roles of physical and biological dispersal barriers in freshwater as well as marine phytoplankton. To address this topic we have used a population genetic approach using AFLP, microsatellite, and recently RADtag high throughput sequencing technology, combined with laboratory experimental studies. By exploring the population genetic structure, we could assess dispersal and diversification among populations. We specifically investigated a freshwater raphidophyte (*Gonyostomum semen*), which has recently expanded its habitat range, and find that the populations are already genetically differentiated. Moreover, we present results showing that hydrological connectivity does not matter on the local scale, while we have genetic isolation with distance. In a marine diatom, on the other hand, large-scale ocean currents and environmental factors shape the populations. We have also explored the role of biological barriers, such as priority effects and local adaptation, in causing population differentiation in phytoplankton.

### 12KN.2

#### BLUE-GREENS OFF BALANCE?

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Elevated pCO<sub>2</sub> may fuel phytoplankton photosynthesis and thereby promote their growth. Particularly

CO<sub>2</sub> fixation in cyanobacteria may be facilitated as they possess a RubisCO (Ribulose-1,5-bisphosphate Carboxylase/Oxygenase) with among the lowest affinities for CO<sub>2</sub>. To compensate for this low affinity, they have developed carbon concentrating mechanisms (CCMs), which are cellular mechanisms to enhance CO<sub>2</sub> concentrations in the vicinity of RubisCO. Various freshwater cyanobacteria species are toxic and can proliferate under eutrophic conditions, forming dense harmful blooms. Among the most common toxins produced are microcystins. Consisting of seven amino acids, these are nitrogen-rich compounds of which many variants exist with distinct toxicities. The development of a cyanobacterial bloom results in the depletion of resources, including CO<sub>2</sub>, light and nitrogen. During bloom development, cells thus experience large shifts in resource availabilities with possible consequences for CO<sub>2</sub> fixation, carbon:nutrient stoichiometry and toxin synthesis. In this talk, I will explore the extent to which elevated pCO<sub>2</sub> may alter both carbon and nitrogen assimilation, and how this may affect the toxicity of freshwater cyanobacteria.

### 12OR.1

#### INORGANIC CARBON ACQUISITION CHARACTERISTICS OF AUSTRALIAN FRESHWATER MICROALGAE FROM A SUBTROPICAL RESERVOIR

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Most microalgae from marine and freshwater environments possess CO<sub>2</sub> concentrating mechanisms (CCMs) that render them relatively unaffected by CO<sub>2</sub> levels in the environment. Nonetheless, different species express CCMs to varying extents and thus future rises in atmospheric CO<sub>2</sub> could potentially influence species composition in algal populations. In an attempt to understand the potential effects of elevated atmospheric CO<sub>2</sub> on algal growth and populations in

freshwater systems we investigated some of the carbon acquisition characteristics of six strains of freshwater phytoplankton from Lake Wivenhoe, South East Queensland, Australia. These included *Cyclotella* sp. (Bacillariophyceae), *Cylindrocapsa raciborskii* (Cyanophyceae), *Monoraphidium* sp. (Chlorophyceae), *Nitzschia* sp. (Bacillariophyceae), *Staurastrum* sp. (Zygnemophyceae), and *Stichococcus* sp. (Chlorophyceae). We determined rates of gross photosynthesis, respiration and the half-saturation rate constants for light ( $K_{0.5, I}$ ) and DIC ( $K_{0.5, CO_2}$ ) by oxygen electrode and rates of electron transport by chlorophyll fluorescence (Walz PhytoPAM). The ability of cells for acquisition of  $CO_2$  vs bicarbonate was measured by isotopic disequilibrium and internal pH, internal carbon pool and the cells' ability to concentrate  $CO_2$  was determined directly via the silicon oil centrifugation technique. Affinities for  $CO_2$  ( $K_{0.5, CO_2}$ ) varied between 0.73  $\mu M$  for the diatom *Cyclotella*, to 5.33  $\mu M$  for the desmid *Staurastrum*. Maximum rates of photosynthesis ranged between 0.55 to 42.6  $nmol O_2 cell.min^{-1}.10^{-6}$  for *C. raciborskii* and *Staurastrum* respectively. Internal pH varied between 7.66 for *Stichococcus*, to 7.97 for *Staurastrum*. We discuss the differences between strains and the consequences for changing populations in a future with elevated atmospheric  $CO_2$ .

## 12OR.2

### ECOLOGY MATTERS: LINKING INORGANIC CARBON ACQUISITION TO ECOLOGICAL PREFERENCE IN FOUR SPECIES OF MICROALGAE (CHLOROPHYCEAE)

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The effect of  $CO_2$  supply seems to play a particularly important role in algal ecology. Inorganic carbon ( $C_i$ ) acquisition strategies are very diverse among microalgae and are influenced by several environmental factors such as pH, light and inorganic phosphorus concentration. Because different species of microalgae vary in their habitat preference, we hypothesized that  $C_i$  acquisition depends on the pH of the preferred natural environment (adaptation) and that the efficiency of  $C_i$  uptake is affected by  $CO_2$  availability (acclimation). Therefore, we analysed four species of green

algae originating from habitats with different pH values for their  $C_i$  acquisition preference by using pH-drift experiments and determining  $C_i$  uptake parameters at different external pH. We additionally studied algae previously adapted to both high and low  $CO_2$  conditions to examine the influence of  $CO_2$  supply. Our results confirmed a significant influence of external pH on growth due to varying pH preferences with pH of the natural habitat as well as on  $C_i$  acquisition in all four species. Final pH values at the end of the pH drifts and  $C_i$ /alkalinity quotients varied among algal species and treatments and therefore led to different efficiencies of  $C_i$  uptake and  $C_i$  species used. Low  $CO_2$  compensation points for all species implied that differences among species are linked to the usage of bicarbonate, which demonstrated that  $CO_2$  concentrating mechanisms were present. In addition, the acclimation from high to low  $CO_2$  conditions during the drift revealed interesting differences of  $C_i$  uptake rates and  $C_i$  uptake efficiencies between species. In conclusion, we found that  $C_i$  acquisition was highly variable within a single phylogenetic group of green algae and showed that there was a connection between  $C_i$  acquisition and ecology.

## 12OR.3

### INDEPENDENT COLIMITATION FOR $CO_2$ AND INORGANIC PHOSPHORUS

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Simultaneous limitation of plant growth by two or more nutrients is increasingly acknowledged as a common phenomenon in nature, but its cellular mechanisms are far from understood. We investigated the uptake kinetics of  $CO_2$  and phosphorus of the algae *Chlamydomonas acidophila* in response to growth at limiting conditions of  $CO_2$  and phosphorus. In addition, we fitted the data to four different Monod-type models: one assuming Liebig's Law of the minimum, one assuming that the affinity for the uptake of one nutrient is not influenced by the supply of the other (independent colimitation) and two where the uptake affinity for one nutrient depends on the supply of the other (dependent colimitation). In addition we asked whether the physiological response under colimitation differs from that under single nutrient limitation. We found no negative correlation between the affinities for uptake of the two nutrients, thereby

rejecting a dependent colimitation. Kinetic data were supported by a better model fit assuming independent uptake of colimiting nutrients than when assuming Liebig's Law of the minimum or a dependent colimitation. Results show that cell nutrient homeostasis regulated nutrient acquisition which resulted in a trade-off in the maximum uptake rates of CO<sub>2</sub> and phosphorus, possibly driven by space limitation on the cell membrane for porters for the different nutrients. Hence, the response to colimitation deviated from that to a single nutrient limitation (Spijkerman et al. 2011 PLoS One 6 (12):e28219).

#### 12OR.4

##### GROWTH AND PHYSIOLOGY OF TOXIC AND NON-TOXIC CYANOBACTERIA IN RELATION TO LIGHT INTENSITY

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Cyanobacteria are major bloom-forming organisms in freshwater ecosystems all over the world. In addition to high concentrations of nitrogen, phosphorus and carbon, light is the most important parameter for algal growth. Different strains within the same species may respond differently in terms of growth and physiology depending on the light climate. To evaluate the growth and physiological parameters of both toxic and non-toxic strains of *Microcystis aeruginosa* and *Anabaena circinalis*, strains were grown at a range of light intensities (10, 25, 50, 100, 150 and 200  $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ ) and their photosynthetic characteristics determined. The study revealed that the toxic strains of both species (for *M. aeruginosa* CS558 and *A. circinalis* CS537 & CS541) require a higher light intensity to saturate growth ( $\mu$ ) compared to the non-toxic strains (*M. aeruginosa* CS338 and *A. circinalis* CS534). Strains maintained at their growth saturating light intensities were then characterised in terms of their basic physiology (light harvesting efficiency  $\alpha$ , light saturation intensity for photosynthesis  $I_k$ , maximum quantum yield  $F_v/F_m$ , relative electron transport rate  $rETR_{\text{max}}$ ). Light-saturated rates of photosynthetic oxygen evolution ( $P_{\text{max}}$ ) and dark respiration ( $R_d$ ) were measured by a Clark type O<sub>2</sub> electrode while  $\alpha$ ,  $I_k$ ,  $F_v/F_m$  and  $rETR_{\text{max}}$  were determined by PAM chlorophyll fluorescence (Walz PhytoPAM). Both  $R_d$  and  $P_{\text{max}}$  showed significant differences between strains with those strains requiring higher saturating light intensity for growth (*M. aeruginosa* CS558 and *A. circinalis* CS537 & CS541) differing significantly from the other strains. Other photosynthetic parameters such as  $\alpha$ ,  $I_k$ ,  $F_v/F_m$  and  $rETR_{\text{max}}$  did not vary significantly

between strains. The results suggest that the different strains are suited to different light habitats. Thus, the temporal and/or spatial changes in the light environmental conditions may affect bloom toxicity by helping to regulate the dynamics of changing dominance and succession patterns of toxic and nontoxic strains.

#### 12OR.5

##### USING CORRESPONDENCE ANALYSIS TO DETERMINE RELATIONSHIPS BETWEEN ABIOTIC FACTORS AND THE DENSITY OF *DESMODESMUS* SPECIES IN POLISH LAKES

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Two oxbow lakes (R1 and R2) and one artificial dam reservoir (R3) were used to evaluate the quantity and diversity range of *Desmodesmus* species occurring during four seasons of one-year. Water samples were quantified for *Desmodesmus* species and identified using the Light Microscope (LM) and Scanning Electron Microscope (SEM). The following water analyses were conducted: "pH, EC, DO, K, Ca, Mg, TN, NH<sub>4</sub>-N, NO<sub>3</sub>-N, TP, SO<sub>4</sub><sup>2-</sup>, Fe and Mn. We used Correspondence Analysis (CA) analysis to determine abiotic factors that influenced the density of *Desmodesmus* species in the three aquatic ecosystems. Not surprisingly, different *Desmodesmus* species inhabited each water body and exhibited different densities of growth, contributing 77% of the total density in R1, 22% in R2 and only 1% in R3. Summer and autumn were the seasons when *Desmodesmus* showed the highest density in the shallow and polymictic oxbow lakes (R1 and R2). In the deep and dimictic reservoir (R3), the highest density of *Desmodesmus* was during autumn. Combining all data into one data-set, four groups were identified. Group 1 (*D. armatus*, *D. quadricauda* and *D. spinosus*) were closely related to conductivity (EC), P<sub>tot</sub> (total phosphorus), chlorides (Cl), phosphates (PO<sub>4</sub>) and Cu. Group 2 (*D. opoliensis* and *D. subspicatus*) were related to Zn, K, water temp, Fe, Mg, SO<sub>4</sub>, suspension and Mn. Group 3 (*D. abundans*) related to ammonia (NH<sub>4</sub>). Group 4 (*D. intermedius*) related to nitrates (NO<sub>3</sub>). These results indicated that each species has specific nutrient requirements (singly or in combination) that affect their density in the water column.

Further research will examine the CA of individual lakes and the response of *Desmodesmus* species to specific nutrient conditions in culture.

## 12OR.6

### SEASONALITY OF INTRA-SPECIFIC CELL SIZE IN THE PHYTOPLANKTON OF LAKE KINNERET

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A distinct pattern of seasonal fluctuations in intra-specific cell size and/or colony size was observed in a large number of phytoplankton species from Lake Kinneret, Israel. The same species showed larger cell size or colony size in late winter and smaller size in late summer, with intermediate sizes in the interim periods. This phenomenon was exhibited by species of chlorophytes, dinoflagellates and cyanobacteria that were abundant enough to be sampled (fortnightly) and measured throughout the year. The annual pattern of fluctuations in size repeated itself over 8 consecutive years (2004–2012). The size fluctuations were independent of the temporal changes observed in cell abundance of each species. Rather, peak sizes coincided with lowest water temperature and highest nutrient availability, minimum sizes with highest water temperature and lowest nutrient availability. These observations fit well with current ecological theory on organism size, where larger organisms occur in colder climates and the reverse in warmer climates.

## 12OR.7

### ON THE EFFECT OF RARE EARTH ELEMENTS ON MICROALGAE: POLLUTANTS AND GROWTH STIMULANTS

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The rare earth elements (REEs = scandium, yttrium, lanthanum and 14 lanthanides) currently are in great commercial demand due to rapid technological progress. In parallel, they are also increasing as environmental pollutants. Our main objective was to study the little known effect of REEs on microalgae. A cyanobacterium and three different microalgae were grown under conditions of exposure to REEs. Toxicity occurred at high concentrations, but at low concentrations, they stimulated microalgal growth rate. We also observed changes in the total amount and specific composition of pigments and lipids. However, the specific growth promoting mechanisms are still unknown. In further experiments, we investigated whether REEs can replace essential metals in their functions and explain growth stimulation in *Desmodesmus quadricauda* (Chlorophyta). We observed that to some extent the REEs could replace calcium but not manganese (where detrimental effects increased). From our results we deduce that despite their relatively low toxicity, it is still difficult to predict the impact of REEs in the environment. Supported by the Long-term Research Development Project no. RVO 61388971 of the Academy of Sciences of the Czech Republic. By the National Program of Sustainability I, ID:LO1416. We thank also the Grant GACR14-00227S of the Czech Science Foundation (GACR) and the Junta de Andalucía y Ministerio de Educación de España (FPU grant AP2009-2770).

## 12OR.8

### SILICA-SCALED CHRYSOPHYTES ON THE SALINITY GRADIENT

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Silica-scaled chrysophytes (Stramenopiles, Ochrophyta) are represented predominantly by freshwater flagellates. The high conductivity group of species was defined by Peter Siver and a diverse silica-scaled chrysophyte assemblage, comprising more than 50 taxa, was previously revealed from brackish waters of the Pojo Bay, the Gulf of Finland, Baltic Sea. However, it was supposed that part of the community was delivered to the area by freshwater river inputs. To investigate the effect of salinity on species diversity



and community structure, we sampled the lakes in the close proximity to the shoreline (up to 5 km) and deep almost closed bays (salinity values of 0.01-2.66 practical salinity units) of the Gulf of Bothnia, Baltic Sea. We excluded exposed seashore sites to minimize river flow impact. Species determination was based on scale morphology, which was investigated with a transmission electron microscope. Salinity was the strongest predictor of diversity; the Shannon index significantly declined with increasing salinity. Community structure of silica-scaled chrysophytes was influenced most strongly by pH. However, pH and salinity were highly correlated. We determined that a pool of species inhabiting the sites with salinity over 0.2 psu represented a subset of the species thriv-

ing in less saline localities. Species that were abundant in low saline sites also were abundant in sites with salinity over 0.2 psu. Twelve recorded silica-scaled chrysophytes (out of 83 taxa) significantly preferred low saline waters. On the contrary, occurrence of six species correlated positively with salinity (e.g. *Mallomonas tonsurata* and *M. alpina*). In contrast to diatoms where a gradually change of species composition on salinity gradient was recorded (number of freshwater species decreased and number of brackish to marine species increased), silica-scaled chrysophytes represent a typical freshwater group where the number of species able to adjust to environmental conditions declined with increasing salinity.

## 13. Omics and genetic resources towards algal domestication

### 13KN.1

#### THE *PORPHYRA UMBILICALIS* GENOME: STUDIES ON THE PATH FROM GENOME TO GROCERY STORE

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An asexual isolate of *Porphyra umbilicalis* (P.um.1) that directly recycles the commercially valuable *Porphyra* blade has been sequenced at the Joint Genome Institute (JGI). The assembled genome is ~96 Mbp (without repeats), with 20,762 predicted genes in a draft assembly. Genic regions average 67% GC. The simple life history found in P.um.1 occurs throughout the northwestern Atlantic, and provides a model for understanding spore differentiation and maturation in a multicellular red alga. To study gene expression associated with production of neutral spores, we assembled 67k transcripts from RNA-Seq analysis on three regions of blades: the central vegetative area (abutting basal rhizoids), the sub-marginal area of the blade where mitotic divisions produce differentiating neutral spores, and the marginal area where there is a cm-deep region of mature neutral spores. In nature, re-wetting of *P. umbilicalis* blades by the incoming tide stimulates release of large quantities of naked neutral spores and accompanying

mucilage. Once settled, neutral spores exhibit amoeboid movement prior to final attachment. Differentiating neutral spores show increased transcriptional activity for some genes associated with photosynthesis. Interestingly, nearly 3,000 transcript assemblies showed upregulation only in the marginal area, including transcripts associated with the cytoskeleton, vesicular transport, and extracellular adhesion. Electron micrographs show increased vesicular activity in marginal spores. These results suggest that mature neutral spores contain a transcriptome that supports spore release, movement to a suitable microhabitat, and substratum attachment. P.um.1 was grown to harvestable size (10-12 cm blade length; 139 (+/- 40) g wet wt/m kuralon line) in Frenchman's Bay (Maine, USA) from such neutral spores. Continued analysis of P.um.1 by our JGI/NSF RCN annotation team should lead to new insights related to its metabolism that will influence product development and enhance our understanding of stress tolerance of this species, its evolution, and its metagenome.

### 13KN.2

#### OMICS IN DECIPHERING THE EVOLUTIONARY WARFARE BETWEEN *PYROPIA* AND ITS PATHOGENS

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As with land crops, algal cultivation beds are suffering from various diseases, ranging from spectacular outbreaks in natural populations, down to significant losses in multibillion dollar crops such as *Pyropia* spp. Recent estimation on economic loss caused by *Pyropia* diseases showed that sea farmers in Korea lose about 10-15 million dollar because of algal disease every year, mostly due to fungal disease caused by the oomycete pathogens, *Olpidiopsis pyropiae* and *Pythium porphyrae*. The recent development of intensive and dense mariculture practices have made some new diseases spread much easier than before. Comparative transcriptomic study showed that the causative agent of green spot disease is a noble RNA virus, which could infect broad spectrum of *Pyropia* species. Host plants intensively use RNA binding proteins for defense against viral infections in nature. We isolated 6 RNA-binding proteins which are highly

upregulated during infection process. Metagenomic studies on *Pyropia* and its epiphytic bacteria showed that some epiphytic bacteria are involved in fungal infection to *Pyropia*. An elicitor released from epiphytic bacteria facilitated zoosporogenesis of *Pythium porphyrae*. The evolutionary warfare between *Pyropia* and its pathogens left many traces in the genomes of both sides. Therefore, proteomic and transcriptomic studies on *Pyropia* and its pathogens are crucial to develop a disease-resistant *Pyropia* strain. The pros and cons of mutation breeding of disease-resistant *Pyropia* strain will be presented.

### 13KN.2

#### GENOME STRUCTURE CHARACTERIZATION OF *SACCHARINA JAPONICA*

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*Saccharina japonica* was regarded as important to the edible human food, alginate production and marine eco-systematic contribution. So far, more than 10 cultivars have been applied in the production in China, but many genomic backgrounds of biological processes and genomic architecture are not understood very well. The genome sequence for the wild *S. japonica* strain was conducted with next-generation sequencing. Genomic DNA libraries were constructed for the subsequent sequenced, which generated approximately 91-fold sequencing reads, and 490 Mb of the assembled genome size. The gene prediction showed that there are a presumed 31,358 genes in *S. japonica* genome. Re-sequencing analysis for the common cultivars and some wild strains were both conducted and compared. The long introns single copy genes were analyzed, and the repetitive sequences of the introns were associated with long repetitive sequences, the correlation between gene intron length and gene expression level were conducted with the expressed genes. The genome sequence data useful to our understanding the metabolic pathways, genetic adaptation and domestication and the good-quantitative varieties formation to the kelp.

### 13KN.4

#### THE DOMESTICATION PROCESS IN THE RED ALGA *GRACILARIA CHILENSIS*: RAPID CHANGES OBSERVED AFTER ONLY 30 YEARS OF INTENSIVE FARMING

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Interestingly, even if the domestication of *Gracilaria chilensis* has begun recently, archaeological evidence suggests that this species was used for food and/or medicine since the first establishment of human populations along the South East Pacific 14,600 years ago. Nowadays, this species is one of the main algal resources in Chile, which is cultivated for agar production. In the 90's, over-harvesting resulted in the collapse of natural stands and stimulated the implementation of farms maintained by fragmentation. A first population genetic study, comparing farmed and wild populations Chilean populations of *G. chilensis*, concluded that farming practices had significantly modified important life history traits in farms. A complementary study, based on temporal variation of genetic diversity in these two types of populations, shows that a mixture of haploids and diploids are always encountered in natural populations that show all the signs of recurring events of recombination. By contrast, most farms are composed only of diploids, generally non-mature, and present genetic variability typical of their clonal reproduction system. New experiments in laboratory-controlled conditions have allowed us to hypothesized that ecological differences between phases and short term reproductive cost are possible trigger of the life cycle changes in farms and that involuntary selection could operate during the ongoing first step of the domestication process. Genome scan approaches are being developed in order to trace the domestication history of this species and to survey the changes in spatial and temporal genetic structure of wild and cultivated populations in Chile.

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## 14. The fate of our marine forests in a changing ocean

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### 14KN.1

#### THE STRUCTURE AND FUNCTIONING OF KELP FOREST ECOSYSTEMS UNDER RAPID ENVIRONMENTAL CHANGE

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Kelp forests dominate shallow rocky habitats across much of the world's temperate coastline. As foundation species, kelps support high levels of primary productivity, magnified secondary productivity, and provide habitat for a highly diverse associated assemblage. However, the abundances and distributions of habitat-forming kelps are responding to global environmental change stressors, including ocean warming, extreme climatic events and the spread on non-native species. Here, I provide an overview of reported kelp forest loss from around the world, with particular focus on climate-mediated changes in ecosystem structure. Further attention is focussed on the northeast Atlantic region, where alterations in kelp forest structure have been recently reported and significant shifts have been predicted. In the southwest UK, for example, the abundance of the 'warm' water kelp *Laminaria ochroleuca* has increased significantly in recent decades, as has the distribution of the invasive kelp *Undaria pinnatifida*. Conversely, the abundances of several northerly-distributed species (e.g. *Laminaria digitata* and *Alaria esculenta*) are expected to decrease under climate change scenarios. The wider implications of shifts in habitat-forming kelp species are poorly understood. Here, I summarise recent research on the likely impacts of shifts in kelp forest structure on local biodiversity and primary productivity. Evidence-to-date suggests that observed and predicted shifts in kelp species distributions may lead to impoverished assemblages in some reef habitats, and altered timings of primary productivity. Greater understanding of the (often subtle) community and ecosystem-level consequences of shifts/replacements of habitat-forming species is needed to better predict the overall impacts of global environmental change.

### 14KN.2

#### STUDYING BIODIVERSITY BY AN INTEGRATIVE APPROACH OF POPULATION GENETICS AND COMMUNITY ECOLOGY: A WAY TO BETTER PREDICT THE FATE OF OUR MARINE FORESTS IN A CHANGING OCEAN?

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Species diversity and genetic diversity have traditionally remained the exclusive domains of community ecology and population genetics, respectively, despite repeated recognition of close parallels between these levels of biodiversity in the literature. Species diversity within communities and genetic diversity within populations are hypothesised to co-vary because of locality characteristics that influence the two levels of diversity via parallel processes (drift, selection and migration) or because of direct effects of one level of diversity on the other. Corroborating these hypotheses, several studies on different taxa from various geographic zones have revealed correlated patterns of species and genetic diversity (mostly positive correlations), suggesting that the SGDC (species genetic diversity correlation) might be a general pattern. In this work, we tested for spatial SGDC patterns in kelp forests along the Brittany coastline (France) by conducting a multi-level biodiversity sampling in 20 localities: in each one, we sampled specimens belonging to the two dominant kelp species *Laminaria digitata* (Hudson) J.V. Lamouroux and *Laminaria hyperborea* (Gunnerus) Foslie as well as macroalgal communities living underneath the canopy formed by these two species. Genetic diversity for these two kelp species was investigated using microsatellites while the species identity of macroalgal communities' specimens was determined based on both morphological and molecular criteria. We tested SGDC for different diversity metrics and at several spatial scales. Our results revealed a majority of positive SGDC, suggesting that genetic diversity within kelp populations and species diversity within macroalgal communities are probably influenced by parallel processes. However, the significance and strength of the correlation varied among the target species, the metrics and the spatial scale



considered. These findings allow highlighting in which cases i) anthropogenic pressures and/or conservation measures have similar effects on species and genetic diversity and ii) one level of diversity can be used as a surrogate for predicting the other.

### 14KN.3

#### COMBINING KNOWLEDGE ON THERMAL NICHES TO SPECIES DISTRIBUTION MODELS IN GEOGRAPHICAL PROJECTIONS IN A CHANGING OCEAN

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Predictions of species distribution are based on projections of the current niches to future climates. The most common method, namely Species Distribution Model (SDM), relies on projecting the realized niche of environmental tolerance in the field, measured by correlating distributional field records with the actual physical conditions. Although a powerful tool, SDM do not explore the mechanistic causes of species shifts, making difficult to predict outside the environmental gradient where the model was trained. In our ongoing research line, we are assessing the projections of SDMs using thermal thresholds suggested by mortality and growth experiments as proxies of the fundamental niches of physiological tolerance of foundation macroalgae: intertidal fucoids and subtidal kelps in current decline in response to coastal warming in southern Europe. A battery of ecophysiological experiments is being performed by: combining physical stressors for testing additive and interactive effects, creating thermal gradients for detecting thresholds and trigger points, manipulating the mean intensity and variance of stress to investigate responses to extreme events, and using lineages from different latitudes for investigating adaptive responses. We use physiological thresholds to produce geographic projections of species' distributions to assess projections obtained using SDMs. The predictions obtained by both approaches are robust in geographic areas where the species shows high or low dominance. Physiological thresholds tend to over-predict the prevalence as they cannot identify absences in climatic conditions within the range of physiological tolerance of the species. SDMs may implicitly capture the mechanisms driving such absences, such as low competitive potential under particular environmental conditions, but this may or may not extrapolate to other geographic areas or times. We present examples of how integrating correlative and mechanistic approaches provides a better understanding of species

distribution and more robust predictions of species distribution under climate change.

### 14OR.1

#### CHANGES IN KELP FOREST BIOMASS AND DEPTH DISTRIBUTION AT KONGSFJORDEN (SPITSBERGEN) BETWEEN 1996/98 AND 2012-2014 REFLECT ARCTIC WARMING

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At our Arctic study site at Hansneset, Kongsfjorden, Spitsbergen we repeated a quantitative diving study of 1996/98 in 2012-2014 in the shallow rocky sublittoral between depths of 0 and 15 m and detected considerable change. There was a 1.7x increase in overall seaweed biomass, and a 4.7x increase in seaweed biomass at shallow depths (2.5 m) between the time periods. The standing stock of the biomass maximum at 2.5 m comprised 14.4 kg fresh biomass m<sup>-2</sup>, a leaf area index of approx. 10 and a very high kelp density, and was composed of three kelp species with a pronounced dominance of *Laminaria digitata* and some *Alaria esculenta* and *Saccharina latissima*. The observed biomass increase indicates an increased productivity, which has been predicted to occur in the Arctic under climate change scenarios. Besides the increase in overall mean biomass, maximal biomass values of perennial seaweed species also increased. At the same time a decrease in (1) the lower depth limit of dominant brown algae by several meter, (2) an uplift of the biomass maximum from 5 to 2.5 m as well as of the upper depth limit of many seaweed species and (3) a general decrease of maximal biomass values of annual species became apparent. This complex pattern is probably a consequence of contrasting effects induced by Arctic warming, namely the reduction of fast ice and ice-scouring in spring as well as a supposedly deteriorating underwater irradiance climate by increased sedimentation with reduced phases of clear water.

**14OR.2****LOSS OF THE HABITAT-FORMING  
*CYTOSEIRA MEDITERRANEA* AT ITS  
NORTHERN-LIMIT OF DISTRIBUTION IN  
THE MEDITERRANEAN SEA**

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Large habitat-forming brown algae are suffering a general decline in temperate seas. In the Mediterranean Sea, cases of loss of habitat-forming *Cystoseira* species (Fucales, Phaeophyceae) are numerous. If the losses are massive for the species living deeper than 1 m depth, little attention has been paid to the shallow species forming narrow belts just below the sea surface (infralittoral fringe), as the Mediterranean endemic *C. mediterranea*. The species is widely distributed in the Mediterranean Sea. In the western basin, its northern limit is along the SW coast of France. We assessed the past and current distribution of *C. mediterranea* in France over the last 200 years. The species is known to only occur in French Catalonia and Languedoc. On the eastern French coasts, the species is replaced by its vicariant *C. amentacea*. The most recent distribution of *C. mediterranea* was mapped in May 2012, 80 km of coast have been surveyed exhaustively and precisely (boating and snorkeling). In French Catalonia, the species is suffering a steady decline through the 20<sup>th</sup> century. From 2003 to 2012, 58 % have been lost. Before 2003, the species was forming dense populations, whereas it was only distributed in patches in 2012. Lost populations were replaced by the opportunistic less-structuring *Corallina* spp., encrusting corallinales and mussels (*Mytilus galloprovincialis*) assemblages. In the absence of increase of anthropogenic local stressors, this recent decline might be due to the exceptional storms that hit the region in winter 2008 and 2010 and the subsequent mussel proliferations. Most worrying is the northern limit extinction in the 1980's at Sète and Agde because of habitat destructions. In the remaining natural stretches of coast, only abundant mussel and *Corallina* spp. assemblages were mapping. Given its natural dispersal capabilities the recovery of *C. mediterranea* in the region appears unlikely.

**14OR.3****CAN WE MONITOR CHANGES IN COASTAL  
HABITATS FROM OUR DESKTOPS?**

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Major changes are occurring in the abundance of large brown seaweeds in the northeastern Atlantic. Some observations of change are anecdotal, although direct evidence of change is increasing. However, one of the major impediments in obtaining reliable evidence is the lack of consistent monitoring over periods of time. The reasons for this are multiple, including changes in personnel, policy and funding, but also cost and inaccessibility of sites. In order to overcome these problems we are developing methods to determine the abundance of intertidal seaweed habitats using a combination of remote sensing and ground truthing. In this talk we compare assessments of coastal habitats based on high quality aerial imagery, freely accessible web-map imagery and high resolution satellite data. We describe our approach using classification methods to estimate and quantify the extent of coastal habitats over time. We present results from two pilot studies in the UK - Thanet, Kent and Isle of Wight, and discuss the implications of this approach in 'real-time' monitoring and use.

**14OR.4****TOLERANCE TO CLIMATE CHANGE OF  
EARLY LIFE-STAGE *FUCUS VESICULOSUS*  
VARIES AMONG SIBLING GROUPS**

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Early life-stage of the bladderwrack *Fucus vesiculosus* is highly influenced by the climate change factors temperature, CO<sub>2</sub> and eutrophication. Intraspecific genetic diversity of Baltic *Fucus vesiculosus* populations is low, compared to e.g. Atlantic populations, which may limit their potential for adaptation. To assess the role of intraspecific genetic diversity on the tolerance towards environmental change we manipulated their diversity: Plots with full-sibling

groups of *Fucus* germlings each originating from one parental pair represents the low diversity level, whereas plots with sibling groups from multiple parental pairs represent the high diversity level. Climate change was simulated according to the year 2100 in the near-natural scenario Kiel Benthocosms by maintaining the environmental fluctuations of the Baltic Sea and adding 5°C warming, 600 µatm pCO<sub>2</sub> and doubling the nutrient concentrations. Germlings responded to warming with higher mortality and enhanced growth rates. High pCO<sub>2</sub> concentrations increased growth due to a fertilisation effect. Non-photochemical quenching was lower under warmed than ambient temperatures. A positive co-tolerance among sibling groups towards warming and acidification indicates the possible attenuation in presence of the multiple factors. Considerable differences among sibling group performance indicate a higher adaptive potential for genetically diverse populations. The high diversity levels also showed higher survival, indicating possible facilitation processes among genotypes. Microsatellite genotyping is in progress for revealing whether and how selection processes took place in high diversity levels. We conclude that impacts on early life-stage bladderwrack depend on the combination of stressors and season and that genetic variation is crucial for local adaptation to climate change stress.

#### 14OR.5

##### TRANSCRIPTOMIC ACCLIMATION IN *DESMARESTIA ANCEPS*

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Brown algae of the order Desmarestiales dominate rocky shores along the Antarctic Peninsula, where they form huge underwater forests providing habitat and nurseries for various marine organisms. Geographical distribution as well as the determination of vertical patterns of macroalgae is constrained by abiotic factors such as light, including UV and temperature. Therefore, global environmental changes might have a negative impact on performance and survival of the Desmarestiales. Whereas the basic

physiological and ecophysiological characteristics of *Desmarestia anceps* are well studied, until now no study is available on the molecular processes underlying acclimation to abiotic stress. Within the framework of this study, we generated the transcriptome of the brown alga *Desmarestia anceps* using the MiSeq sequencing technology and tested for differential gene expression in response to different light and temperatures conditions using RNASeq and the HiSeq 2500 sequencer. We established a reference transcriptome consisting of 20.2 million paired end reads, out of this, a total of 53,745 assembled transcripts were generated, with contig length ranging from 301 bp to 37,509 bp. For investigating gene expression profiles of sporophytes were exposed for 24h to combinations of light intensities, UV radiation and temperatures (2, 7, 12°C). The highest numbers of differential expressed genes were found in response to the 12°C treatments, indicating that high temperatures are more harmful for *Desmarestia anceps* than low temperatures, resulting in stronger efforts to overcome the negative effects. *Desmarestia anceps* responds to abiotic stress with a multitude of transcriptional changes. Expression profile changes in several metabolic pathways including e.g. nucleotide metabolism, lipid metabolism, and amino acid metabolism were observed. UVR stress in general caused induction of nucleotide & lipid catabolism. A critical component of molecular acclimation mechanisms to high temperature stress in *Desmarestia anceps* is the induction of protein and lipid modification processes for maintaining membrane and protein function.

#### 14OR.6

##### RECOVERY OF LARGE BROWN ALGAE FORESTS AFTER DESTRUCTIVE FISHERY: ECOLOGICAL RESTORATION INSIGHTS

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Marine forests of large brown algae are disappearing worldwide due to several human impacts, such as coastal urbanization, marine pollution and alteration of sedimentation rate. Proliferation of herbivores, often due to overfishing of predators or destructive fisheries, is another major cause of loss of marine forests. In this study, we assessed for the first time, the recovery of forests in an area highly impacted by the illegal fishing of the date mussel *Lithophaga lithophaga*, one of the most harmful human activities affecting subtidal rocky habitats. We tested the role of the limited dispersal of *Cystoseira* species and the potential effect of herbivores on *Cystoseira* recovery by crossing these two factors in a multifactorial orthogonal experiment in the field. Our experiment showed that the natural recovery of marine forests can be low and seems to be limited by both the low dispersal and by the presence of high densities of herbivores, in part a consequence of the destructive fishery. Ecological restoration of marine forests should be considered in particular cases, such as the date mussel destructive fishery (together with protection measures preventing poaching). Following our results, restoration actions should consider seedlings and may therefore depend on the presence of reproductive individuals in the vicinity of the area to be restored. Major herbivores (both sea-urchins and fish) should be excluded or regulated at least in the first phases of restoration protocol. As stressed in previous work, the priority for management of coastal ecosystems should always be the conservation of existing forests, by the institution of MPAs and the implementation of targeted monitoring.

#### 14OR.7

#### NEW METHOD FOR RESTORING DEGRADED CYSTOSEIRA FORESTS

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The demise of large, long-lived species can lead to major seascape shifts. Such shifts have been widely observed in sublittoral habitats affected by environmental degradation, involving the disappearance of kelps and other engineering species of seaweeds. Members of the genus *Cystoseira* (Fucales) are important seascape makers in the Mediterranean, but are currently experiencing a severe decline as a consequence of environmental degradation. Conservation and recovery of their populations depends not only on the persistence of adults, but also on the successful recruitment and development of early life-history stages. However, recruitment of this genus is very limited, impeding the recovery of decimated populations, even when the impact that led to their regression has ceased. Here we assay new techniques for restoring degraded *Cystoseira* forests by artificially increasing recruitment. Two different restoration techniques have been tested. The first one (in situ seeding) consisted in collecting fertile apical branches from a natural population and placing them on a new locality. The second technique (ex situ seeding) was similar but the fertile apical branches were placed on laboratory seawater tanks; *Cystoseira* recruits were cultured at the laboratory and transplanted to natural populations when they reached a height of 1 cm. The initial density of settlers was significantly higher for the in situ seeding than for the ex situ seeding. However, those differences greatly decreased one year after the beginning of the experiment, with greater mortalities on the in situ experiment. Two years after the restoration experiments, all *Cystoseira* populations were more or less bell-shaped, and symmetric, peaking between 2-5 cm size classes, and with maximum heights of 13 and 12 cm in the populations restored with the in situ and the ex situ method, respectively. Both



techniques are low cost and low time consuming and might be applied for restoring threatened populations of *Cystoseira* spp.

#### 14OR.8

### SLOW FLOW HABITATS AS REFUGIA FOR CORALLINE ALGAE FROM OCEAN ACIDIFICATION

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Ocean acidification (OA) is the ongoing decline in the pH of surface waters, and the projected 0.4 unit pH decrease by 2100 is likely to negatively impact benthic coastal organisms that fabricate calcium carbonate ‘skeletons’. Coralline algae are considered the most vulnerable of all calcifying organisms to ocean acidification. Research has focussed on identifying susceptible species but we also need to identify refuge habitats to enable their protection. The susceptibility of coralline algae to OA depends on the pH at their surface, and this is regulated by the interplay between algal metabolism and water motion. Algal metabolism causes local increases (photosynthesis, nitrate uptake) and decreases (calcification, respiration, ammonium uptake) in the pH at the algal surface; pH at the surface of a seaweed can thus fluctuate by  $\pm 0.5$  units on a daily cycle. Water motion sets the thickness of the diffusion boundary layer, and thick DBLs have been shown to ameliorate the negative effects of ocean acidification on coralline algae via their action of retaining metabolically produced high-pH seawater at the algal surface, thereby preventing dissolution of calcium carbonate. Seawater velocities within beds of canopy forming seaweed, including members of the orders Laminariales and Fucales, are much reduced (75-95%) compared to the mainstream seawater velocity. Such slow-flow habitats may thus provide

natural, low-cost refugia for coralline algae from ocean acidification. Aside of studies of “Tintenstriche” (= dark pigmented epilithic phototrophic communities on rock cliffs), records of endolithic Cyanobacteria from the European Alps are rare. We found a microbial community inhabiting an episodically dry-fallen surface of a waterfall tufa in the Alps, forming one or two distinct black to dark blue-green permanent endolithic layers in 1-2 mm and 4-6 mm below surface, and at a distance of 1-4 m from water-run areas. The waterfall tufa forms from ambient (non-thermal) waters during episodes of increased runoff. Community structure showing a prevalence of Cyanobacteria was studied by culture-independent and culture-dependent approaches and covered light-and electron microscopy (TEM) and molecular characterization using 16S rRNA-based clone libraries. Clonal inserts were screened by restriction fragment length polymorphism and individual restriction types were sequenced. The limestone was studied in epoxy-impregnated, petrographic thin sections under polarized light- and dark-field microscopy. Although there is a relationship of the endolithic community to the surface-dwelling “Tintenstrich” microflora, we found a smaller number of taxa within the endolithic layers, which seem specifically adapted to this microenvironment. The endolithic microbial communities presumably consist mainly of limestone-dissolving taxa rather than bio-calcifying taxa. The sub-millimeter sized pore spaces (parallel underneath the outer calcified surface of the waterfall tufa) they inhabit is filled with damp air rather than water; this enables the community to withstand extended periods of drought as typical of central-alpine valleys during summer. The functional groups of Cyanobacteria in the endolithon are composed of, both, nitrogen fixers (*Dichothrix* sp.) and non-N-fixers (*Aphanothece* sp.), adapted to low-light conditions; in the epilithon high-light and desiccation-tolerant taxa were recorded (*Gloeocapsa alpina*, *Gloeocapsopsis* sp., *Chondrocystis* sp.). *Nostoc* sp., however, was common in the cultures from both habitats.

## 15. Algae and Signalling - regulation of processes from cell to globe

### 15KN.1

#### LIPID SIGNALING IN PLANKTON COMMUNITIES

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Zooplankton organisms release chemical compounds into the surrounding water that induce pivotal changes in phytoplankton prey. Few of these signaling molecules have, however, been identified. Here we discuss the role of polar lipids as signals in the marine pelagic exemplified by a group of novel signaling lipids, the copepodamides. Copepodamides constitute at least 12 closely related taurine conjugated lipids released by copepods. Harmful alga respond to pico-, to nanomolar concentrations of copepodamides by inducing defensive traits such as production of saxitoxins (paralytic shellfish toxins), and changes in morphology. Copepods have species-specific copepodamide composition allowing for high specificity in interactions with responding organisms. We present exudation rates, dose response relationships for prey organisms, depth resolved distribution of copepods and copepodamides in coastal water, as well as the composition of copepodamides in common NE Atlantic copepods. Copepods are ubiquitous in aquatic habitats and copepodamide signaling may consequently have large-scale effects in pelagic food webs. Finally, we argue that polar lipids may be an important group of signals based on the copepodamides and additional recently described lipid signals from the marine pelagic.

### 15OR.1

#### EXPRESSION OF A PKSIII GENE AND SOLUBLE PHLOROTANNIN SYNTHESIS IN RESPONSE TO ABIOTIC AND BIOTIC STRESSES IN THE BROWN ALGA *FUCUS VESICULOSUS*: CONSTITUTIVE VERSUS INDUCTIVE PROTECTION

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Brown algal phlorotannins are structural analogues of terrestrial plant condensed tannins and similar to plant phenols that carry numerous bioactive functions. These molecules are largely studied in the chemical ecology of Phaeophyceae because of their interesting activities including as antioxidant and anti-microbial, anti-appetant and bio-adhesive capacities. However, phlorotannin biosynthetic pathways have been hardly characterized at the molecular level. We found that a predicted type III polyketide synthase in the *Ectocarpus* genome, EsiPKS1 heterologously expressed in *E. coli*, catalyzes a major step in the biosynthetic pathway of phlorotannins, i.e. the synthesis of phloroglucinol monomers using malonyl-CoA. Phlorotannins are then formed by the condensation of phloroglucinol units, however, the following steps in this

biosynthetic pathway are still unclear. The confirmation of the first step has been used in order to better understand the mechanisms that regulate this metabolism in brown algae. By combining integrated approaches of gene expression, quantification and profiling of soluble phlorotannins, we have shown that these metabolites ensure the constitutive protection in *Fucus vesiculosus* exposed to UV-B radiation and could also be induced very early during grazing. The development of these specific molecular tools opens some new perspectives in ecophysiological and ecological studies of the functions of brown algal phenolic compounds.

## 15OR.2

### INNATE IMMUNITY REGULATES THE EXCRETION OF ANTI-SETTLEMENT COMPOUNDS BY *FUCUS VESICULOSUS* AND OTHER BROWN SEAWEEDS

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Oligoguluronates are known as pathogen-associated molecular patterns that activate oxidative burst responses and induce defense-related protein expression in sporophytes of Laminariales. In contrast, most other brown seaweeds – for example members of Fucales or Dictyotales – were up to now considered as incapable of oligoguluronate recognition, as oxidative burst responses after challenge with this signal are usually not observed. Here we show that exposure of *Fucus vesiculosus* and *Sargassum muticum* to oligoguluronate results nonetheless in a defensive response that targets microbial settlers. At natural concentration surface extracts from elicited specimens of both species were significantly more deterrent to bacteria than surface extracts from unelicited specimens. Since this effect was not observed 1 h after challenge with oligoguluronate, but observed 1 – 3 d after elicitation, it was suggested that it was an induced rather than an activated response. A targeted analysis of established deterrent compounds from *F.*

*vesiculosus* revealed that fucoxanthin and proline concentrations are higher on surfaces of elicited specimens, while DMSP concentrations are unaffected by this treatment. In the case of fucoxanthin, increased surface concentrations were correlated with decreased tissue concentrations, which hints at an induced translocation of fucoxanthin from chloroplasts to the algal surface within 24 h after oligoguluronate recognition. We conclude that oligoguluronate must be perceived like a pathogen-associated molecular pattern in members of the Fucales, inducing a non-specific deterrent response against microbial settlers. We also present results from a comparable study on *Taonia atomaria*, a member of the Dictyotales.

## 15KN.2

### A CHEMICAL ARMS RACE MEDIATES HOST-VIRUS INTERACTIONS DURING ALGAL BLOOMS IN THE OCEAN

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Marine viruses that infect marine microorganisms are recognized as major ecological and evolutionary driving forces, shaping community structure and nutrient and energy cycling in the marine environment. Nevertheless, the cellular mechanisms that govern these host-virus dynamics are largely unexplored. *Emiliana huxleyi* is a globally important coccolithophore forming massive algal blooms in the North Atlantic Ocean that are routinely infected and terminated by large DNA viruses, coccolithoviruses (EhVs). This unique host-pathogen interaction spans more than 12 orders of magnitude, from the individual cell ( $\sim 10^{-6}$  m) to large algal blooms ( $\sim 10^6$  m). We therefore aim to bridge this gap by investigating both the intricate cellular mechanism induced during host-virus interactions as well as studying the mechanism of viral transmission over the large scale of algal blooms. We explore the molecular basis for host-virus dynamics and the signal transduction pathways that mediate host resistance and susceptibility to viral infection. Major efforts in our lab are focused on the role of sphingolipid and ROS metabolism and their function in host PCD and autophagy during viral replication strategies. The high prevalence of viral-encoded metabolic enzymes from aquatic systems strongly points to their central role in shaping the evolutionary 'arms race' between marine microbes and their viruses, and consequently, the flow of carbon in marine food webs

## 15OR.3

**SIGNALLING IN BACTERIAL-MACROALGAL SYMBIOSIS: CHEMOTAXIS IS INVOLVED IN BIOFILM FORMATION BY *ROSEOBACTER* SP. AND *ULVA* MUTABILIS (CHLOROPHYTA)**

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The marine macroalga *Ulva mutabilis* (Chlorophyta) is developing into callus-like colonies consisting of undifferentiated cells with abnormal cell walls under axenic conditions. From the associated microbial flora, *Roseobacter* sp. (MS2) and *Cytophaga* sp. (MS6) are able to induce the complete algal morphogenesis *via* morphogenetic compounds forming a tripartite community. Interestingly, the motile *Roseobacter* sp. establishes a biofilm around the primary rhizoid cells of *Ulva mutabilis*. Using this standardised tripartite model system, we are now able to address various aspects on the cross-kingdom cross talk. We tested the hypothesis if the biofilm formation would be mediated by the attraction of the bacteria through chemotaxis-inducing algal signals. Therefore, a bioassay-guided approach was developed using different solid phases for extraction covering a wide range of polarity. The bioassay was based on the attraction of *Roseobacter* sp. towards algal extracts in a capillary chemotaxis assay. We isolated two biologically active fractions by hydrophobic and hydrophilic interaction chromatography. The analysis of the hydrophilic fraction by HILIC-MS (hydrophilic liquid interaction chromatography coupled to a mass spectrometer) revealed dimethylsulfoniopropionate (DMSP) as both a possible chemotactically active and bacterial growth-promoting factor, whereas the factor, extracted by the hydrophobic solid phase, is still unknown. To obtain a holistic picture of the dynamics of DMSP in the tripartite community, we quantified the dissolved amount of DMSP in the *Ulva* growth medium and performed uptake experiments of deuterium-labelled DMSP with *Roseobacter* sp. under minimal medium conditions. The intracellular DMSP amount indicates a rapid uptake along with a fast metabolism by *Roseobacter*. Besides the chemotactic activity of DMSP, the growth of *Roseobacter* is supported. In summary, the study provides information about the DMSP-mediated physiological relationship between a *Roseobacter* species and the green macroalga *Ulva mutabilis*, and elucidates the role of the algae in shaping this mutualistic relationship.

## 15OR.4

**ENVIRONMENTAL VOLABOLOMICS: DECIPHERING THE CHEMICAL LANGUAGE THAT SHAPES AQUATIC HEALTH**

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All natural systems emit biogenic volatile organic compounds (BVOCs) that can affect atmospheric processes and climate. It is also likely that volatiles carry information on the physiological status of the emitter and that they alter physiological functioning and behaviour across trophic levels in aquatic food webs. Our understanding of the ecology and physiology of key volatiles such as isoprene (C<sub>5</sub>H<sub>8</sub>) and dimethyl sulfide (DMS; (CH<sub>3</sub>)<sub>2</sub>S) is steadily growing. However, the signature qualities of the entire suite of BVOCs produced from single individuals to entire ecosystems, the volatile metabolome or 'volabolome', and its sensitivity to disturbance are poorly explored. Using gas chromatography, we characterised the volabolomes of four freshwater genera (*Chlamydomonas*, *Cyclotella*, *Cryptomonas*, *Aphanizomenon*), before quantifying the volabolomes of the marine dinoflagellate *Symbiodinium* sp. and the diatom *Thalassiosira pseudonana* at two temperatures. Principal Component Analysis (PCA) demonstrated clear separation of algal genera based on their volabolome signatures with significant differences in the first and second principal component scores across genera (ANOVA; PC1,  $F_{3,8} = 38.8$ ,  $P < 0.001$ ; PC2,  $F_{3,8} = 8.2$ ,  $P < 0.01$ ). All taxa could be separated based on principal component one scores (Tukey's HSD,  $P < 0.05$ ) other than *Chlamydomonas* from *Aphanizomenon* which had significantly different principal component two scores ( $P < 0.05$ ). The volabolomes of *Symbiodinium* sp. and the diatom *Thalassiosira pseudonana* grown at 26 °C were different after a step-wise temperature increase to 31 °C over 5 days. Much of this difference was driven by a significant increase in isoprene and DMS. Our investigations demonstrate that algal volabolomes are taxon-specific and affected by environmental conditions. Deciphering this chemical language is an important challenge that could help understanding the regulation of processes from cell to globe.



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## 16. Special Session: PHYCOMORPH

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### 16KN.1

#### EXPLORING BACTERIA-INDUCED MORPHOGENESIS IN THE GREEN MACROALGA *ULVA* (CHLOROPHYTA): A COMBINED CHEMICAL AND GENETIC APPROACH

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Growth, development and morphogenesis of *Ulva* depend on its associated bacteria. Axenic algal germ cells develop just into callus-like colonies consisting of undifferentiated cells. Interestingly, the complex natural bacterial community from *Ulva mutabilis* (Føyn) can be replaced by just two isolated strains, *Roseobacter* sp. and *Cytophaga* sp., which recover the complete morphogenesis applied to axenic gametes. Using this standardized *tripartite community*, our research aims to explore the (i) signal-mediated cooperative interactions between kingdoms, (ii) the biofilm formation at the algal holdfast and (iii) the role of bacteria in algal germination and morphogenesis. The identification of potential growth-stimulating and morphogenetic bacterial substances is hereby essential to the understanding of signal mediated cross-kingdom interactions within the chemosphere of *U. mutabilis*. To unravel the chemosphere two major approaches are followed in analytical chemistry: (i) bioassay guided fractionation and (ii) unbiased metabolite profiling of the exo-metabolome based on GC/MS and LC/MS analyses after solid phase extraction. It turned out that the exo-metabolome is directly influenced by environmental stimuli including the bacterial community associated with *U. mutabilis*. The consistent combination of classical bioassays and explorative analytical methods revealed novel insights into the complex networks of infochemicals and signal molecules along with their ecophysiological significance. However, the underlying molecular mechanism of changes in morphologies needs further investigations preferentially by utilization of a working genetic system. As a result, the transformation of *Ulva* by vector plasmid integration into the genome was developed (W. Oertel, T. Wichard, A. Weissgerber, Journal of Phycology, in revision): The chromosomal *Ulva-RbcS* gene was used for designing a dominant selective marker cassette for shuttle

vectors introduced efficiently in *Ulva* gametes for expression of GFP or insertion mutagenesis. Due to the methods developed, *U. mutabilis* is now well suited to model studies in developmental biology. Reference: Wichard, T. (2015) Frontiers in Plant Science 6:86.

### 16KN.2

#### PLANT HORMONES AND REPRODUCTION IN RED SEAWEEDS: FROM THE CHEMICALS TO TRANSCRIPTOMICS

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An overview will be given of the advances in our understanding of the intervention of plant hormones in the reproductive events of the red seaweeds, that will be linked to the main tasks of the Phycomorph COST ACTION (FA1406). The development of reproductive structures of red seaweeds and their sporulation are affected by the polyamines and the gaseous hormone ethylene. As cystocarp matured, polyamines putrescine (Put), spermidine (Spd) and spermine (Spm) were accumulating, favoring the development of cystocarps. Particularly Spm promoted sporulation in several rhodophytes like *Hydropuntia cornea* (formerly *Gracilaria cornea*) and *Grateloupia imbricata*. Exogenous ethylene favoured the increment in tetrasporangial branches and cystocarps in the red seaweeds *Pterocladia capillacea* and *G. imbricata* respectively. The synthesis of polyamines starts with the decarboxylation of ornithine by the ornithine decarboxylase (ODC, EC. 4.1.1.17) to produce putrescine, which is then transformed in spd and spm by adding groups from S-adenosyl methionine SAM. In *G. imbricata*, the gene *GiODC* has been shown to be involved in cystocarp development, and it also has been shown that its expression seems to depend on spatial and temporal regulation that is associated with the progress of cystocarps, as the number of *GiODC* transcripts abruptly decreases after cystocarp maturation. The synthesis pathways of the polyamines and ethylene, thus share the same precursor SAM. In recent years, different molecular approaches (iPCR, NGS) have been used to understand molecular mechanisms in red seaweed reproduction. Particularly, we have focused

on: (i) how far the elicitors involved in cystocarp development affect to the level of *GiODC* expression, (ii) unravelling of the pathway(s) and the components of signaling for different elicitors involved in the seaweed reproduction, and (iii) the functional analysis of transcriptome of *G. imbricata* with particular emphasis on detecting candidate genes potentially involved in metabolism of growth regulators.

### 16KN.3

#### **COMPARATIVE ANALYSIS OF *CAULERPA* AND LAND PLANT TRANSCRIPTOMES: IMPLICATIONS FOR KAPLAN'S ORGANISMAL THEORY**

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Convergent morphologies have arisen in plants multiple times. In non-vascular and vascular land plants, convergent morphology in the form of roots, stems, and leaves arose. The morphology of some green algae includes an anchoring holdfast, stipe, and leaf-like fronds. Such morphology occurs in the absence of multicellularity in the siphonous algae, which are single cells. Morphogenesis is separate from cellular division in the land plants, which although are multicellular, have been argued to exhibit properties similar to single celled organisms. Within the single, macroscopic cell of a siphonous alga, how are transcripts partitioned, and what can this tell us about the development of similar convergent structures in land plants? Here, I will present a *de novo* assembled, intracellular transcriptomic atlas for the giant coenocyte *Caulerpa taxifolia*. Transcripts show a global, basal-apical pattern of distribution from the holdfast to the frond apex in which transcript identities roughly follow the flow of genetic information in the cell, transcription-to-translation. The analysis of the intersection of transcriptomic atlases of a land plant and *Caulerpa* suggests the recurrent recruitment of transcript accumulation patterns to organs over large evolutionary distances. Our results not only provide an intracellular atlas of transcript localization, but also demonstrate the contribution of transcript partitioning to morphology, independent from multicellularity, in plants.

### 16KN.4

#### **SUCCESSFUL SEAWEED AQUACULTURE BASED ON FUNDAMENTAL BIOLOGICAL KNOWLEDGE - WHAT ARE THE MAIN CHALLENGES?**

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Meeting the demands for food and energy from a global population growth of 2 billion people, reaching 9 billion before 2050, will require millions of tons of new biomass resources. The European seaweed industry has recently expanded by the establishment of several companies aiming for cultivation and processing of seaweed biomass for a commercial market. There are, however, needs for both, cost effective cultivation methods for production of the seaweed biomass in predictable quantity and quality, as well as new processing methods that ensure valuable compounds or products to be derived from the raw material preferably in a way that enables a complete utilization of the biomass. An industry based on processing of cultivated seaweed requires a stable and predictable delivery of biomass with defined qualities. The cultivation biology has to be developed to guarantee this, but there are still many challenges and limitations that need to be solved before the seaweed aquaculture can meet this demands. Some important biological challenges are: 1. Biofouling on the cultivated seaweed restricts the cultivation period and diminish the commercial value of the biomass for use as human consumption and industrial applications. 2. The processing industry requires a predictable content of demanded components in seaweed (carbohydrates, proteins, minerals, anti-oxidants and other high valuable components), but due to seasonal and environmental conditions in the growth period, large variations are found. 3. New species with demanded properties such as fast growth rate, low biofouling activity and valuable chemical content are attractive for aquaculture, but cultivation protocols need to be developed. 4. Viral, bacterial and fungal diseases may cause problems in this emerging aquaculture industry and measures to enhance the resistance against seaweed diseases will be required.

### 16KN.5

#### **MACROALGAL DEVELOPMENT AND MORPHOGENESIS: DEPLOYING A NEW INTERNATIONAL INITIATIVE TO ADVANCE KNOWLEDGE AND TRANSFER**

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Macroalgae develop macroscopic bodies through a high diversity of processes including the regulation

of cell division and cytokinesis, cell-cell communication, diffusion of endogenous signalling molecules, complex chemical interactions with bacteria, and the integration of diverse environmental cues. Beside this complexity rivaling with that of other major macroscopic organisms like land plants, the number of studies dedicated to this field is limited, making the current knowledge about the precise developmental processes in macroalgae very incomplete. In addition to having macroalgal basic sciences hanging back, progresses in seaweed aquaculture are impeded, as current bottlenecks for an improved and cost-effective seaweed biomass production require a deeper and more specific knowledge of macroalgal development

to be solved. Therefore, combining the skills of phycologists and plant biologists all involved in basic and applied studies of macroalgal development, the recent European COST network PHYCOMORPH. ([http://www.cost.eu/COST\\_Actions/fa/Actions/FA1406](http://www.cost.eu/COST_Actions/fa/Actions/FA1406)) aims at developing and coordinating this research field so that significant progresses are made which allow future accurate and cutting-edge development of modern aquaculture techniques. The talk will present PHYCOMORPH and illustrate its objectives through a few examples of current studies as highlighted in the recent research topic of *Frontiers in Plant Science* (<http://journal.frontiersin.org/researchtopic/2598>).

## Manton session

### MANTON.1

#### BIODIVERSITY, SECONDARY METABOLOME AND ECOLOGICAL ROLE OF FUNGAL ENDOPHYTES ASSOCIATED WITH THE BROWN ALGAE *LAMINARIA DIGITATA*, *ASCOPHYLLUM NODOSUM*, *SACCHARINA LATISSIMA* AND *PELVETIA CANALICULATA*

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Morphologically-complex brown algae are colonized by endophytic fungi that do not cause apparent damage on their host. In plants, endophytes protect their host against biotic and abiotic stress, presumably through the production of metabolites. However, in macroalgae, the study of the diversity and the role of endophytic fungi associated with seaweeds are still largely unexplored. In this context, we determined the diversity of culturable fungal endophytes in *Laminaria digitata*, *Ascophyllum nodosum*, *Saccharina latissima*, *Pelvetia canaliculata*. 121 fungal strains were thus isolated from algae sampled in France and Scotland, which grouped into more than 40 molecular operational taxonomic units. Selected marine ascomycete strains such as *Dendryphiella* spp were chemically studied and new fungal metabolites were isolated and characterized. In order to evaluating the potential role of fungal endophytes within the symbiosis relationship with their algae host, activity of these fungal metabolites against pathogens of brown algae was tested (*Eurychasma dicksonii*, *Anisoplidium ectocarpii*, *Maullinia ectocarpii*, *Pseudomonas alginovora*, *Pseudoalteromonas bacteriolytica*). Some of them exhibited potent antioomycete or antibacterial activities against pathogens suggesting a plausible ecological role

in these understudied fungi-algae mutualistic relationships. We are now exploring this concept further *via* the development of tripartite bioassays, where the interaction between the endophyte and some known pathogens will be measured *in vivo*.

### MANTON.2

#### TWO-PHASED CELL POLARISATION IN THE BROWN ALGA *DICTYOTA*

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In most complex eukaryotes development commences with the establishment of cell polarity that determines the first axis of the future body plan. The underlying mechanisms of polarity establishment are still emerging. Using a combination of microscopy, pharmacological experiments and transcriptomic analyses we show that cell polarity in the brown alga *Dictyota* is established in a two-phased process with the first phase narrowing down the possible polarisation vectors to a set of two. Like in land plants, the zygote of *Dictyota* establishes the apical-basal axis during the first cell division. Upon egg activation, the zygote undergoes an F-actin/myosin dependent, 90s lasting elongation along a maternally determined axis that is reflected in the cytoplasmic distribution of plastids. Which of the two poles of the resulting prolate spheroidal zygote will acquire the basal cell fate, is determined environmentally as assessed by the direction of unilateral light. The second phase is accompanied and dependent on zygotic transcription instead of uniquely on maternal factors. In that embryogenesis in brown algal plant systems is more similar to higher plants rather than animal system. Cell polarisation as observed in *Dictyota* whereby determination of direction and sense of the polarisation vector are mechanistically and temporally decoupled, in two distinct processes is unique.



**MANTON.3****THE IMPORTANCE OF REVEALING CRYPTIC DIVERSITY IN RELATION TO ASSESSING THE STRUCTURAL INTEGRITY OF THE MAERL BED HABITAT**

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Rhodoliths, non-geniculate coralline red algae that can exist as free-living fragments, are an important habitat-forming (maerl) group. Around the UK they are the major calcified habitat formers, with maerl beds found in south-west England (Falmouth) and around Scotland. Due to the structural and functional complexity of these organisms, they support a high level of biodiversity. Their susceptibility to climate change makes them ideal candidates to study the effects of ocean warming and acidification. However, little research has focused on their structural integrity and whether, in future CO<sub>2</sub> conditions, they will be able to continue to support a high level of biodiversity. Using Finite Element Modelling, previous research employing 2D models suggested that under future CO<sub>2</sub> conditions coralline algae were more prone to fracture. Here we compared 3D geometric cubes representing various coralline algal features with biologically accurate models derived from computed tomography scanning. In doing so, we have been able to show that these 3D cubes can accurately represent the coralline algal skeleton. Results reiterated that future CO<sub>2</sub> conditions will cause these skeletons to be more prone to fracture. With responses to climate change being species-specific, it is important to have an understanding of the species within these maerl beds. Hence, an identification study was performed using molecular techniques on specimens from the Fal (south UK) and Berwick upon Tweed and Oban (north UK). Results revealed a new species of *Lithothamnion* in the north and it was concluded that there was no evidence of *Lithothamnion lemoineae*, which had previously been recorded in the UK. We can now tailor our models to be species-specific and therefore make accurate predictions as to how the structural integrity of these rhodoliths will be affected in future global change and consequently the impact this will have on the organisms that are supported within the habitat.

**MANTON.4****MULTIDISCIPLINARY ANALYSIS OF A FRESHWATER LAKE MICROBIAL COMMUNITY UNDER DIFFERING NUTRIENT REGIMES**

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In 2012, a report from the Algal Biotechnology Special Interest Group identified a wide range of ways in which algae could be converted to biofuels and bioenergy. The report identified bioremediation as a feasible and cost effective source of algal feedstock. Therefore, we aimed to gain a better understanding of bioremediation targets such as natural microalgal blooms. The goal was to gain insights into large-scale production of algal communities for bioenergy as well as helping to understand whether harvesting from the natural environment is a feasible approach for feedstock generation. The aim of our study was to undertake a multi-parameter analysis of a microcosm simulating a freshwater lake under bloom conditions. An artificial bloom was induced in 15 L microcosms, which were inoculated with an environmental sample and grown in constant light and temperature using a freshwater lake-simulating medium. Measurements of water quality (pH, temperature, DO), chemistry (NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub><sup>-</sup>, PO<sub>4</sub><sup>-</sup>) and biomass (chlorophyll, cyanobacteria, bacteria) were undertaken. We applied a multivariate statistical approach to examine the role of water quality and nutrients on the spatiotemporal structure and composition of functional microbial groups. We coupled this with a metagenetic survey of population composition and a metaproteomic study, which provided a signature of ecosystem function. Together with multivariate analysis, 'omics technologies provide an understanding of community composition and a signature of ecosystem function. The interpretation of the community profiles provided enhanced understanding of bloom formation for predictability (restoration goal) and control (cultivation goal).

**MANTON.5****MATING TYPE RELATED GENES IN PSEUDO-NITZSCHIA MULTISTRATA**

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Sexual reproduction is a fundamental phase in the life cycle of diatoms, linked to the production of

genotypic diversity and the formation of large-sized initial cells that ensure population persistence. It occurs only within cells below a certain threshold size and, in heterothallic diatoms only between strains of opposite mating types. We aim at identifying genes involved in mating type determination in the marine planktonic diatom *Pseudo-nitzschia multistriata*. This species is recorded in coastal waters worldwide and produces the neurotoxin domoic acid. A reference genome has been generated and transcriptomes have been produced for strains of opposite mating type (MT+ and MT-). Differential expression analysis provided a list of putative MT-linked transcripts that were then validated with qRT-PCR using independent strains. Four MT-related genes were identified, two over-expressed in MT+ and two over-expressed in MT-. The expression pattern of the candidate genes was followed in a 24 hours time course experiment to verify whether they were regulated in dependence of light or cell cycle phases. Experimental evidences demonstrated their involvement during mating recognition in early stages of sexual reproduction while genetic analyses excluded that they could be the master gene responsible for mating type determination. Expression of the candidate genes has been analyzed also in samples below and above the sexualization size threshold (SST). Functional analysis is ongoing to understand their role in the chemical communication occurring between opposite mating types. Elucidating the molecular and genetic basis of MT determination and sexual reproduction in diatoms will contribute to a better understanding of the regulation and evolution of their life cycles and reproductive strategies. Results from this study could also provide molecular markers to trace the distribution of MT+ and MT- cells in environmental samples.

#### MANTON.6

##### DIVERSITY AND PHYLOGENY OF THE DINOFLAGELLATE GENUS *TESTUDODINIUM* (DINOPHYCEAE)

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The genus *Testudodinium* was established in 2012, comprising of two species formerly classified as *Amphidinium* and one novel species. Currently these

three species of benthic dinoflagellates remain the only described species in the genus. During our research, we have isolated 14 strains that have been assigned to this genus based on morphological observations and phylogenetic analyses. These strains were isolated from samples from seabed sampling sites in sub-tropical waters of the Kagoshima and Okinawa prefectures, Japan. In this presentation, we shall elucidate the diversity of the genus *Testudodinium*. The strains fall into four major clades within *Testudodinium*, three of which contain a described species. The fourth clade consists of four species that are characterised by a hypocone with a rugose dorsal surface and by the presence of a unique internal feature, props, which are distributed throughout the cell, spanning the height of the cell. The clade including *T. maedanese* does not consist of the single described species but forms a species complex of morphologically indistinguishable strains. The clade containing the two strains described as *T. corrugatum* contains two novel species. None of our strains appear to be related to the type species *T. testudo*, either morphologically or phylogenetically. In conclusion, we show that the genus *Testudodinium* is far more diverse than the described species indicate.

#### MANTON.7

##### NUTRIENT AND LIGHT RESPONSES IN TWO ESTUARINE RHODOPHYTES: IMPLICATIONS FOR THEIR ZONATION

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This study examines how photoacclimation and nutrient uptake responses can affect the zonation of *Bostrychia scorpioides* and *Catenella caespitosa*, two epiphytic red macroalgae that grow spatially segregated in the narrow intertidal zone of Palmones estuary (South of Spain). Uptake rates of ammonium, nitrate and phosphate and changes in photosynthesis- and growth-irradiance curves were measured at concentrations and light levels found in the field. *Bostrychia scorpioides* presented higher uptake rates than *C. caespitosa* for N-sources, especially for  $\text{NH}_4^+$ , the most abundant N form in the estuary. On the contrary, *C. caespitosa* had a greater capability for phosphate uptake. Turnover rates estimated from internal N and P and nutrient uptake rates suggested a slower renewal of nutrients in *B. scorpioides* than in

*C. caespitosa*. Photoacclimation experiments showed that both species had maximum photosynthetic rate ( $P_m$ ) and photosynthetic efficiency ( $\alpha$ ) at 20–40  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ . *Bostrychia scorpioides* had higher  $P_m$ ,  $\alpha$ , dark respiration rate and light compensation point ( $E_c$ ) than *C. caespitosa* at all acclimation irradiances. Growth-irradiance curves revealed a higher  $E_c$  value for growth in *B. scorpioides* than for *C. caespitosa*, which agreed with the irradiance at their respective distributional limits in the field. While light clearly restricts the growth of *B. scorpioides* to the uppermost intertidal zone, it would not prevent *C. caespitosa* from growing at saturating irradiances. However, *C. caespitosa* is absent at the upper intertidal zone where that high light occurs. At the uppermost level of the intertidal, the longer periods without nutrient supply could limit the performance of *C. caespitosa* to a certain extent, whereas *B. scorpioides* would be more able to cope with the reduced nutrient availability given its greater independence on external supply. Both species have distinct capacities for acquiring nutrients and different light requirements, which partially explains their intertidal zonation.

#### MANTON.8

##### BACTERIAL DIVERSITY CHANGES TO SIMULATED LOCAL AND GLOBAL STRESSORS ON THE CANOPY-FORMING ALGA *CYSTOSEIRA COMPRESSA*

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Canopy-forming brown algae provide structurally complex habitats to a variety of species, along temperate rocky coasts. This includes rich but understudied microbial communities, triggered by availability of protected micro-niches and large amount of organic carbon. Canopy-forming algae are retracting worldwide particularly in urban areas, where they tend to be replaced by low-lying, turf-forming algae. Recent works suggests that nutrient enrichment (typical of many coastal regions) interact synergistically with other local and global stressors accelerating this shift, but the underlying causes of these effects are unknown. Epiphytic bacteria can functionally regulate and support the productivity and the resilience of macroalgae. Therefore, we hypothesised that the loss of canopy-forming algae might be mediated by

changes in the epiphytic microbial communities in response to interacting local and global stressors. We used next generation sequencing (Illumina, Miseq) to characterise the epiphytic bacteria associated to the canopy-forming alga *Cystoseira compressa*, as this is one of the most relevant canopy-forming algae in the low intertidal shores around Italy. We also carried out a factorial experiment to investigate the combined effects of nutrient enrichment (local anthropogenic stressors) and heat stress (climate change-related stressors) on the survival, growth and photosynthetic rate of *C. compressa* and the diversity of associated microbial communities. Results showed a significant increase in OTU richness with increasing of algal surface age. Moreover, there was a clear difference between the microbial composition associated with macroalgae and that of the surrounding seawater. Finally, in situ experiment showed an effect of nutrient enrichment and heat stress on both photosynthetic activity and bacterial community composition.

#### MANTON.9

##### INVESTIGATING THE FEASIBILITY OF HIGH VALUE COMPOUND PRODUCTION IN MICROALGAE

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Microalgae have recently been shown to hold significant promise as a novel biotechnological production platform. A commonly used model organism is the green microalga *Chlamydomonas reinhardtii*. Previous studies have mainly focused on the production of recombinant proteins, but not much has been done to investigate the feasibility of high value compound production in microalgae. We want to explore the potential benefits and limitations of microalgae for biotechnological applications. Terpenoids are a large class of high value natural products. They are an attractive target for engineering, as they often cannot be synthesised chemically due to their complexity. To date, these compounds usually are extracted from higher plants at low yields. Producing them in microalgae could be a sustainable and cost-effective alternative. In the algal cell, recombinantly expressed

enzymes can use abundant precursor molecules for light-driven product formation. In a first proof-of-concept study we used a recently developed low-cost chloroplast transformation protocol to express a 91 kDa bifunctional terpene synthase. This enzyme catalyses the formation of a diterpene that can potentially be used as a precursor molecule for fragrance production. In this study we obtained homoplasmic transformants which produced promising amounts of recombinant protein.

#### **MANTON.10**

#### **USE OF HETEROTROPHY AND MIXOTROPHY FOR ALGAL BIOMASS PRODUCTION**

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Considerable research has been carried out on the potential for algal-derived biofuels, yet this has not yet been made a commercial reality due to the high cost and low biomass productivity of algal biofuels. Research conducted so far has mainly focused on

optimizing phototrophic cultivation of algal biomass. However, the maximum algal biomass one can achieve using phototrophically grown algae is restricted by light or inorganic carbon availability, and sometimes both. Therefore, other modes of growing algae such as heterotrophy and mixotrophy need to be investigated. We grew *Scenedesmus* sp. in complete darkness using molasses as the growth medium and found that heterotrophy led to bigger cells, faster growth rates and denser cultures as compared to phototrophically grown cultures. Moreover, these heterotrophically grown cultures surprisingly retained their photosynthetic apparatus with similar values in all the photosynthetic parameters measured. Exposing molasses-grown cultures to light during the late exponential phase further boosted growth rate and biomass production significantly. As a result, the biomass obtained through this process (mixotrophy) is twice as large as with heterotrophically grown cultures and three times larger than with photosynthetically grown cultures. We also observed some interesting changes in the photosynthetic apparatus under heterotrophic and mixotrophic conditions that allow the cells to keep their photosynthetic rates on par with photosynthetically grown cultures. Furthermore, calculations suggest that the molasses addition to growth media will only cost ~ 1 cent per liter. Overall, this study provides promising leads for a greener future.