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Copies of the abstracts will be distributed among registered delegates
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The abstracts are arranged in alphabetical order by first author, presenting authors are underlined.

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ANALYSIS ON SEaweEDS *Kappaphycus alvarezii* Familiarity, Green and Brown Varieties Using Random Amplified Polymorphic DNA Method

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The seaweed *K. alvarezii* is a common macroalgal species throughout South East Asia waters. Both the green and brown varieties are economically important in Indonesia. Random Amplified Polymorphic DNA (RAPD) was used in this research to obtain information about familiarity rate between green and brown Asia varieties. The aim of this study was to obtain information about genetic data of species that can be used for seaweed farming. Familiarity and specific marker between the two varieties, green and brown, were analyzed on samples from Pinrang. The genetic gap between samples among locations was taken from Lombok, Madura and Pinrang. The isolation of DNA was done using modified of phenol- chloroform method with five primers genomes for PCR amplification. The data were processed by RAPDistance Package Software Version 1.04 and supported by Unweighted Pair Group Method of Arithmetic (UPGMA), in a package with Sequential, Agglomerative, Hierarchical and Nested Clustering (SHAN), aimed at grouping the resulting data based on genetic gap. The result showed that the genetic gap of green and brown seaweed have smaller rate compared to location rate. Familiarity between Pinrang green and brown seaweeds shows a smaller rate (genetic gap average of 0.63). The genetic gap of green seaweed from the three locations, Lombok, Madura and Pinrang, was 0.765 (with range: 0.53–0.85), while the genetic gap of brown species based on location points was 0.64 (with range: 0.2–0.83). This genetic gap also remains smaller compared to that of green and brown species from three locations, precisely at 0.705 (with range: 0.180–0.88).

PHYCOTECHNOLOGY FOR ENVIRONMENTAL MONITORING AND SUSTAINABILITY

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Phycotechnology is the application of algae for human welfare through manufacturing or service industries. It refers to the technological applications of micro and macro-algae. It includes the uses of algae in the fields of agriculture, aquaculture, production of Food, feed and value added materials, energy, healthcare, environment, genomics, proteomics, biologicalresearch, nanobiotechnology, bionanotechnology etc. The ecosystem services provided by diazotrophic cyanobacteria (blue-green algae) by converting atmospheric (molecular) nitrogen into bio-accessible nitrogenous compounds, is an example for Natural Phycotechnology. Use of algae by human beings for benefits dates back to centuries. Many species of Cyanobacteria like *Nostoc*, *Arthrospira (Spirulina)* and *Aphanizomenon* had been used as a food for human survival during the early centuries. Many microalgae are used as excellent sources of food and feed due to their easy digestibility and nutrient content. Algae like *Chlorella* and *Spirulina* are used as SCP. Species of *Dunaliella* provide richest source of high quality products like glycerol, carotenoids, proteins etc. Most recently *Euglena gracilis* has been identified as a source of nutrients like essential vitamins, minerals, amino acids, fatty acids and other nutrients and a unique non-digestible material Beta-1, 3-glucan which will serve as an efficient fibrous component. The algal-produced proteins showed biological activity comparable to the same kind of proteins produced by traditional commercial techniques. Therefore the microalgae can be considered as the best cellular bio-factories for the production of fine biochemicals and therapeutic substances. Currently micro-algae are being exploited for environmental protection as many species of *Chlorella*, *Chlamydomonas* and *Scenedesmus* etc. have demonstrated their ability for selective uptake, accumulation and biodegradation of pollutants and thus help in remediation. They are used in biological reclamation of sewage since they can immobilize heavy metals from aquatic systems. Microalgae can also be used as biocontrol agents like 'Insect' a commercial bio-insecticide prepared from the dead biomass of diatom frustules. Microalgae are of significant use in healthcare. Chlorellin from the green microalga *Chlorella* is an effective antibiotic against Gram +ve and -ve bacteria. Algae are an excellent feed stock for

green fuel as they are used for the production of biodiesel, bioethanol, biogasoline, biomethanol, biobutanol and biohydrogen due to their least environmental impact. Many species of diatoms are being explored for synthesizing nanoparticles and for developing nanoscale drug delivery systems. Automated Particle Analysis by EDS carried out on the diatom frustules collected from the mangrove area of Muscat, Oman has shown the presence of nanoparticles of O, C, K, Ti, Al, Si and Zn.

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NITROGEN-RELATED TRANSPORTERS AT WORK ON POTASSIUM DEFICIENCY AND SALT TOLERANCE IN *PYROPIA YEZOENSIS*

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Seaweeds have developed a unique strategy to survive in a high-salinity environment (approximately 400 mM Na). By contrast, the growth of a model land plant, *Arabidopsis thaliana*, is retarded at 100 mM Na or above in the media. Our data indicated that extremely high Na concentrations and decreased K concentrations in the plant body were observed for *A. thaliana* exposed to 100 mM Na whereas a red alga *Pyropia yezoensis* growing in seawater maintained relatively low Na and high K concentrations. In order to understand the molecular mechanisms which allow *P. yezoensis* to withstand the marine environment, cDNA libraries derived from multiple stages in the life cycle were created. These libraries were expressed in a heterologous system and the candidate genes involved in K deficiency and salt tolerance were screened. The list of candidates included multiple genes encoding nitrogen-related transporters such as an ammonium transporter (*PyAMT1*), a nitrate transporter (*PyNRT2*) and a urea transporter. Expression of *PyAMT1* and *PyNRT2* in an *E. coli* strain deficient in K uptake dramatically improved growth on low K and/or high Na. Addition of ammonium to the media also improved growth in K deficiency. *PyAMT1* was found to be expressed 20,000 times more in gametophytes over sporophytes whereas *PyNRT2* expression was high in both life stages. Upon overexpression of *PyAMT1* in *A. thaliana*, tolerance to K deficiency was enhanced. Taken together, our data suggest that increased nitrogen contributes to K deficiency and salt tolerance in plants.

THE EFFECTS OF GRAZING ON THE MICROBIOME OF AN INVASIVE BROWN SEAWEED (*SARGASSUM MUTICUM*)

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The effects of grazing on terrestrial plants, and how they respond to the attack, have long been studied. It is now becoming clear that the entire holobiont (host genome+microbiome) has a role in induced resistance, through the production of secondary metabolism and/or by shifting bacterial community composition from “housekeeping bacteria” to “protective” microbes. Such mechanisms are poorly understood in macroalgae. Some studies have shown that grazers may have a preference for native seaweeds over invasive ones. *Sargassum muticum* is a successful invasive seaweed on European coasts where it has been present for over 40 years and its “ability to avoid predation” has been advanced as one of the reasons for this success. In this exploratory study, we used 16S amplicon sequencing and metagenomics to investigate how the microbiome of *S. muticum* shifts under grazing attack and how that is reflected in the structure and functional profile of associated bacteria. Preliminary results show that the bacterial community structure and predicted functions shift towards specific taxa that are related to secondary metabolite production and oxidative stress responses. With increasing algal aquaculture, a systems biology framework becomes important to understand the role of associated bacteria in seaweed establishment success. By understanding the mechanisms behind this community, it is possible that induced resistance of associated bacteria could be manipulated for biological management in aquaculture to replace chemical treatments.

ON THE DIVERSITY OF PLANKTONIC CHLOROPHYTES FROM THE MUSEUM LAKE, GOVERNMENT BOTANICAL GARDEN AND ZOO, THIRUVANANTHAPURAM KERALA, INDIA

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A diversity study of the planktonic Chlorophycean members has been carried out in the Thiruvananthapuram Museum Lake and a total of 54 species have been recorded over a period of two years (February 2013 – January 2015). *Chlorella* sp. was found throughout the study period. *Scenedesmus* was recorded as the major taxon with nine different species, *Scenedesmus quadricauda* and *S. dimorphus* were the dominant of those species. The Shannon-Wiener diversity index for the study period showed a good diversity of the species for all seasons (2.9–3.27). The Canonical Correspondence Analysis (CCA) between environmental variables and the dominant chlorophytes indicated an influence of the physical and chemical parameters on their distribution in the Museum Lake.

EPIPHYTIC LOAD ON INTERTIDAL HALODULE UNINERVIS FROM ONE OF THE HOTTEST REGIONS ON EARTH: EFFECT OF DISTANCE FROM SHORE

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The north of the Arabian Gulf region frequently registers the highest global summer temperatures (>51°C). During low tide, intertidal flora are directly subjected to these extreme air temperatures and must be tolerant, or otherwise perish. The intertidal flora of Kuwait include epiphytic algae and cyanobacteria attached to the seagrass *Halodule uninervis*. These epiphytes on long blades of the seagrass were

compared for biomass and diversity between two intertidal locations. The first is Doha, situated in the semi-enclosed Kuwait Bay and characterized by longer exposure periods. The second location is Dhbaayah at the southern coastline, open to the Arabian Gulf, characterized by shorter exposure periods. In addition, the effect of the proximity to the shoreline was also examined, as the upper intertidal is more exposed to desiccation than the lower. The epiphytic load, which was mainly the pennate diatom *Cocconies*, on Doha's *H. uninervis* was around three times higher than in Dhbaayah. Also, in both locations, the mid intertidal blades had a greater epiphytic load (115.8 × 10² and 32.5 × 10² *Cocconies* biovolume mm², respectively). Is it possible that these epiphytes help seagrass to retain moisture during low tide and therefore promote survival? Additional investigation is required to prove such a role.

IDENTIFICATION OF CYANOBACTERIA IN A EUTROPHIC LAGOON ON THE SOUTHERN BALTIC COAST

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Cyanobacteria are found in habitats worldwide and have different sizes, forms and features. Picocyanobacteria such as *Synechococcus* and *Prochlorococcus* dominate the oligotrophic oceans. The eutrophic shallow brackish waters around the Baltic Sea are dominated by other picocyanobacteria, which mostly form colonies. These colony builders are usually determined for monitoring purposes using morphological features of the colonies. However, the huge plasticity of these features requires the application of molecular genetic methods. A biphasic approach of culture dependent and culture independent methods was applied to samples of the Darß-Zingst Bodden Chain, a eutrophic inner coastal lagoon on the southern Baltic sea. The 16S rRNA gene phylogeny of isolated strains as well as clone sequences indicated a strong dominance of *Cyanobium* species. Using morphology, *Aphanothece* dominated the phytoplankton with up to 90% abundance. The discrepancy between morphologically determined *Aphanothece*-like species and genetically determined *Cyanobium* may be found in other eutrophic and shallow habitats, too. This

bias has consequences for indicator species identification in monitoring programs of such systems and therefore for analysis of long term monitoring data.

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EFFECT OF ATMOSPHERIC CO₂ INCREASE ON PIGMENT CONCENTRATION AND COMPOSITION OF DIFFERENT MARINE PHYTOPLANKTON SPECIES

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Ocean acidification produces significant changes in phytoplankton metabolism that can affect growth and primary production. This study shows the effect of atmospheric CO₂ increase on growth rates and pigment concentration and composition of three marine phytoplankton species with global relevance as marine primary producers: the diatoms *Thalassiosira pseudonana* Hasle & Heimdal and *Skeletonema costatum* (Greville) Cleve and the coccolithophorid *Emiliania huxleyi* (Lohmann) W.W.Hay & H.P. Mohler. The species were aerated during at least 3 weeks with current concentrations of atmospheric CO₂ (400 ppmv CO₂) and with concentrations expected for future scenarios of climate change (1,000 ppmv CO₂), under conditions that promoted an acclimated and steady basal metabolism to the experimental CO₂ concentrations, and under conditions that promoted an activation of the basal metabolism after a perturbation. The results showed that the CO₂ increase produced higher growth rates in *T. pseudonana* and *E. huxleyi* under acclimated conditions but showed opposite trends in the three species under perturbed conditions when the basal metabolism departed from the steady state. Concomitantly, in acclimated *S. costatum* and *E. huxleyi* pigment concentrations decreased significantly under high CO₂ and steady state acclimated conditions, but the response was reversed when the metabolism was perturbed. The deepoxidation state of cyclic xanthophylls (DEPS), that has been shown to be related to photoprotection, also showed opposite patterns between both metabolic conditions, and was significantly lower in *T. pseudonana* and *E. huxleyi* under high CO₂ and steady state acclimated conditions. In conclusion, the results show that the atmospheric CO₂ increase affects pigment concentration and

composition of marine phytoplankton. The responses are in agreement with a down-regulation of the intracellular pool of metabolites, including those related to photoprotection, under high CO₂ and acclimated steady-state conditions and an up-regulation under perturbed conditions, with important consequences on growth rates.

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HISTORICAL MORPHOLOGICAL CHANGES OF THREE SPECIES OF GELIDIALES

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Recent studies have determined an increase in the sea surface temperature around the Canary Islands Archipelago, of 0.25 to 0.5°C per decade since the 1970s. However, the local consequences of this warming on macroalgae communities remains unknown. The Gelidiales *Gelidium canariense* and *G. arbusculum* are endemic species of the archipelago, whereas *P. capillacea* has a worldwide distribution. These species are ecosystems engineers and directly compete for space with each other. The aim of this study was to determine the effect of this SST increment on the morphology of these species. To do so we used deposited specimens of the TFC Phyc herbarium (Universidad de La Laguna) from 1970 to 2015.

We established two periods of temperatures, cold (from 1970 to 1989) and warm (1999 and 2015), for statistical purposes and then analyzed morphological changes of the three species over these four decades. The morphological variables analyzed were: (1) length of the greatest primary branch of all specimens, (2) length of the primary branch and of the greatest secondary branch for tetrasporophytes, as well as (3) the number of apices and (4) reproductive structures (tetrasporangial sori) of the primary branch. A significant shrinkage of the greatest primary branch of all specimens of the two endemic species, *G. canariense* and *G. arbusculum*, was detected for the warm period. Asexual individuals of both species also showed a reduction in

length of the primary branch. The length of the greatest secondary branch also showed a decrease at high temperatures for the three species, but more sharply in *Gelidium* species. The number of apices was only significantly reduced for the warm period in *G. arbusculum*. Finally, *G. canariense* and *G. arbusculum* showed fewer reproductive structures per primary branch during the warm period. These results clearly show the deleterious effect of warming on endemic species.

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UNRAVELING THE CORE AND VARIABLE BACTERIAL COMMUNITIES OF EVOLUTIONARILY DISTANT MARINE MACROALGAE

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Marine macroalgae are multicellular photosynthetic organisms that are important determinants of the health of marine ecosystems. The thalli of these marine algae harbor complex bacterial communities that are known to influence several key aspects of the life cycle, physiology and defence mechanisms of the algal host. Macroalgae are classified as red, green and brown depending on the color imparted by pigments to the thallus and the three groups are evolutionarily distant from each other. Even though the considerable influence of the bacterial community on the algal host is well recognized, there is limited knowledge about the core bacterial community that is uniformly present in all three groups. Also, it is not known if or how the composition of the total bacterial community differs across the three groups and with regard the species, tissue-type, environment or geographical distribution. We set out to answer these questions by studying the bacterial communities present on the three algal lineages represented by *Sargassum natans*, *Gracilaria* sp. and *Caulerpa racemosa*. A total

of 71 samples from the three algal species were collected from the Atlantic Ocean and the Caribbean Sea around Puerto Rico. For identifying the core bacterial community, we studied the bacterial community on and inside the algal thallus and compared it with the bacterial community in the water from the immediate surroundings of the algae and with water collected at a distant location from the algae. The V4 hypervariable region of the 16S rRNA gene was targeted for Illumina sequencing and the resulting data were processed using QIIME. The initial set of 3,738,440 reads underwent de-replication as well as quality filtering and resulted in 1,291,607 sequences and 3,024 operational taxonomic units (OTUs). Our results identified core bacterial communities present in all three algal species collected from different locations which may suggest their conserved role across taxa. We also found significant class- and genus-level differences between different tissues of algal samples suggesting an inter-tissue diversity of bacteria in algal tissues. Our results will provide a good foundation for further studies into identifying and assigning functions to the core bacterial communities across various algal lineages.

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STABILITY AND BIOSORPTION OF ALGAL BEADS IN REAL INDUSTRIAL WASTEWATER

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Algae and alginate, are considered biosorbents because of the carboxyl and hydroxyl groups in their structure. Therefore, algal (*Scenedesmus obliquus*) alginate beads, as ecofriendly biopolymers, have been used in the present work to optimize a bioremediation system suitable for industrial wastewater (w.w.). Different alginate suspensions containing CaCO₃, and another polymer were solidified in BaCl₂ or CaCl₂ to determine algal bead size, algae and w.w. change in 48 different treatments. The best algal bead

structure was transferred to industrial w.w. to investigate metal absorption in real and 25% diluted w.w. by ICP. Also, alginate beads without algae, free algae and a control have been considered in the present work. Results showed that, in all cases, chitosan has a negative effect on beads resistance and algae growth.

Interestingly, CaCO₃ makes a small safe environment for algae to grow and increased bead resistance and size, more than those of other beads. In all experiments, although titration in BaCl₂ reduced algae population, it caused more stable structure than that of CaCl₂. In some cases, algae released into w.w. and turned it to green, while beads stayed green or white. Various combinations of ions in w.w. cause restriction in ion exchanges during the first hours of biosorption, but after 48h, ion exchanges started in alginate and algal beads. Immobilized and free algae grew better in diluted w.w. than that in complete w.w. In the first few days of experimentation, Ba and Ca were released from beads into the w.w., but started to be absorbed after 4 days. Free and immobilized algae absorbed Fe and P better other metals, while alginate shows high potential for absorption of all ions in different amounts.

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FAME PRODUCTION AND FATTY ACID PROFILING OF MICROALGAE FOR BIODIESEL PRODUCTION

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Identification along with quantification of neutral lipids is an important aspect in terms of biodiesel production from microalgae. Different techniques have been explored for identification and quantification of neutral lipids. Amongst which Gravimetric, Gas chromatography mass spectrometry (GC-MS) and Nile red cell staining are the popular methods for lipid quantification. Such quantification is necessary for studying lipid production by microalgae, particularly in microalgae carbon capture and biodiesel production set ups. In present investigation, direct transesterification reaction is performed which eliminates

extraction step resulting in rapid analysis of algal free fatty acid (FFA) and triacylglyceride (TAGs) using gas chromatography after esterification to their corresponding fatty acid methyl esters (FAMES). Transesterification reaction was performed under acid catalyzed condition in the presence of methanolic solution in order to achieve the complete conversion of fatty acids into their respective FAMES. Five algal species i.e. *Chlorella*, *Chlorococcum*, *Chroococcus*, *Nostoc* and *Calothrix* was investigated in order to explore their potential of lipid production from biomass cultivated on a laboratory scale. Quantification of fatty acid using gas chromatography is a fast and robust process allowing multiple analysis of samples. In this investigation it was confirmed that direct transesterification is highly accurate method for quantifying total fatty acid extracted from the above mention algal strains.

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COMPARISON OF DIRECT AND INDIRECT TRANSESTERIFICATION FOR FAME PROFILING IN CHLOROCOCCUM SP.

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Microalgae can survive in diverse environmental conditions, capturing carbon dioxide from the atmosphere and converting it into more complex organic molecules such as lipid. Lipid is preferably synthesized during stress conditions. Oleaginous green microalgae are considered a potential feedstock for biofuel production. Commercialization of biofuel from microalgae can be achieved only by enhancing algal biomass and its lipid content. The biggest challenge in the field of biofuel production is the efficient extraction of lipid from selected species and its transesterification. In the present investigation, direct and indirect methods of transesterification were compared in the microalga *Chlorococcum*, which was grown in a BG 11 medium, at 3,000 lux light intensity and a temperature of 30 ±3°C with a shaking speed of 160 rpm. Indirect transesterification was found to be a more suitable method for the extraction of lipids and conversion to corresponding methyl esters. The yields of C16 (21.85%), C18:2 (24.18%), and C18:3 (18.81%) fatty acids was maximal under indirect transesterification.

REDISCOVERY OF THE OCHROMONAS TYPE SPECIES, OCHROMONAS TRIANGULATA (CHRYSOPHYCEAE), FROM ITS TYPE LOCALITY (LAKE VEYSOVE, DONETSK REGION, UKRAINE)

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Ochromonas is a large, polyphyletic genus with approximately 125 described taxa. The type species, *Ochromonas triangulata*, was described by Vysotskii in 1887, but it was never reported again. We re-collected in the type locality, Lake Veysove, Ukraine (48° 52.046' N, 37° 37.533' E), and established four cultures of *Ochromonas triangulata*. Cell morphology, including the triangular shape, as well as cell division, palmelloid stage, and cyst structure generally agreed with Vysotskii's description. Molecular phylogenetic analysis based on the 18S rRNA gene revealed 14 clades of *Ochromonas*-like flagellates including the clade anchored by *O. triangulata*. We also conducted a concatenated analysis using the 18S rRNA and the rbcL genes, and we recovered the same 14 clades. The clade contained the strains CCAP 933/27 and CCMP1861, previously named *O. tuberculata*, which were re-identified as *Chrysastralla paradoxa* and *C. breviappendiculata*, respectively. The clade that included the well-known *Ochromonas danica* was assigned an older genus *Chlorochromonas*. Another clade included the *Poterioochromonas* strains, but we were unable to convincingly connect species names to the strains because authentic strains were unknown or not examined. The new generic name *Melkoniania* gen. nov. was proposed for a distinct clade of marine flagellates. These changes addressed some of the issues associated with the polyphyletic *Ochromonas* sensu lato.

MOLECULAR PHYLOGENY AND MORPHOLOGY OF SOME DIATOMS FROM THE FAMILY PINNULARIACEAE
D.G. MANN

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Morphology and molecular phylogeny of *Envekadea* Van de Vijver, Gligora, Hinz, Kralj & Cocquyt species and a new genus *Metzeltinia* Andreeva, Maltsev, Kulikovskiy gen. nov. from Indonesia were analyzed.

Morphology and ultrastructure of two new *Envekadea pseudocrassirostris* strains are presented and discussed. Studied samples were collected from a brackish lake in Indonesia.

A new marine benthic diatom genus *Metzeltinia* was identified from light and electron micrographs. The most characteristic morphological feature of this genus is the structure of the biseriata striae. It includes one new species: *Metzeltinia indonesica* Andreeva, Maltsev, Kulikovskiy, Podunai sp. nov., distributed in Indonesia, southeast of Sulawesi island.

In the present report we offer new data based on a molecular investigation of *Envekadea* and *Metzeltinia* and remarks on its systematic position within the raphid diatom phylogenetic tree. In a two-gene (18S rDNA fragments and partial rbcL gene) phylogenetic analysis *Envekadea* and *Metzeltinia* were most closely related to members of the genera *Pinnularia* Ehrenberg and *Caloneis* Cleve. It is proposed to place the genera *Envekadea* and *Metzeltinia* in the family Pinnulariaceae. The results are discussed in the context of the morphological similarities and differences between these genera and their closest phylogenetic allies.

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GLOBAL CLIMATE CHANGE AND LOCAL EXTINCTION OF CANOPY BROWN ALGAL SPECIES IN HONG KONG, CHINA

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Myogropsis myagroides and *Sargassum siliquastrum* are common brown algal canopy species in the temperate northwest Pacific. Their range extended all the way south to subtropical Hong Kong. These two species formed the dominant canopy in the subtidal region of Long Lok Shui, Tung Ping Chau Marine Park in Hong Kong at depths of 2 to 5 m. *Myogropsis myagroides* was not found outside the marine park but *S. siliquastrum* was more extensively distributed in several sites outside the marine park. In the last 20 years, *M. myagroides* disappeared, around the year 1999. Its disappearance was triggered by an unusually high frequency of five storms of >100 km /hr in 1999 that severely disrupted its reproductive phenology, leading to recruitment failure. The population never recovered.

The more extensive *S. siliquastrum* bed in the marine park was decimated in 2008 by urchin overgrazing. To date, almost 10 years after, no sign of recovery is in sight. *Sargassum* beds in sites outside the marine park, notably in Lo Fu Ngan, remained healthy from 2008 to 2013. Low sea urchin density in this site due to commercial harvesting was thought to be the reason for the maintenance of this *Sargassum* bed. Recently, however, this *Sargassum* bed also completely disappeared. Its disappearance seemed to be triggered by unusually warm winter in the last five years, which delayed the peak reproductive season. This also led to the subsequent recruitment failure.

In both cases, unusual weather conditions disrupted the phenology of these populations. Recruitment failure was ensured either because the reproductive output was much reduced as in the case of *M. myagroides*, or that recruits were produced off season in warm summer, rather than during the colder spring as in the case of *S. siliquastrum*. The latter was compounded by sea urchin overgrazing. Warmer winter also meant active grazing by sea urchins all year round, which otherwise would have slowed down during late fall and winter during the peak growth period of the canopy species.

“KNOWN UNKNOWN” IN THE MOLECULAR PHYLOGENY: WHERE FOSSIL TAXA MIGHT HELP US RESOLVE THE EVOLUTIONARY HISTORY OF DIATOMS

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Far from resolving the issues and controversies of diatom systematics and classification, using phylogenies based on molecular data (such as DNA sequence data) has reignited old discussions about the monophyly of the “centric” and “araphid pennate” diatoms. However, as more families are represented in the taxon sampling and more DNA markers are used, stable patterns in the resulting phylogenies are starting to persist across the different analyses, such as the monophyly of groups with well-defined morphological synapomorphies such as the Coscinodiscaceae, Thalassiosiraceae, Eupodiscaceae or Bacillariophyceae. What also persists is that the relationships between these well-defined groups, such as within the Mediophyceae, are still poorly-resolved. Additionally, there are several taxa or small groups of taxa, such as *Ellerbeckia*, *Proboscia*, *Toxarium* and *Stictocyclus*, which are on long branches and poorly-resolved within the phylogenetic trees, where additional DNA sampling is simply not possible based on our current knowledge of diatom diversity. Extinct taxa likely fill these spaces in the phylogenetic tree where additional DNA data seem unlikely to be found. In this presentation, we discuss where in the phylogenetic tree of diatoms these “known unknowns” can be found. We also propose some genera, both living and extinct, for an expanded analysis of morphological characters to potentially fill the gaps in the tree based on molecular characters.

EFFECT OF ENVIRONMENTAL FACTORS ON SELECTED MICROALGAE STRAINS FOR EPA AND DHA PRODUCTION

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Microalgae are considered a promising source of various high-value products with health benefits. They have the advantages of not containing cholesterol, having a favorable image among consumers and being able to be cultivated in bioreactors with inexpensive substrates: sea water, CO₂, solar energy and minerals. Specifically, microalgae synthesize and produce large amounts of omega-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFA), with eicosapentaenoic (EPA, 20:5 n-3) and docosahexaenoic (DHA, 22:6 n-3) acids being of particular interest. This project focused on microalgal n-3 LC-PUFA as a sustainable alternative to the traditional fish oil production. Investigations focused on the relative influence of specific abiotic factors (i.e. light, temperature and nutrients) on microalgal biochemical composition, and determined optimum culture conditions to reach a balance between omega-3 productivity and efficient growth rate. Eight algal species were selected according to their potential for favourable EPA and DHA production. Microalgae were batch-cultivated in triplicate at four different temperatures (8, 14, 20 and 26°C), with constant light (100 μmol.m⁻². s⁻¹), over a period of 14 days. Growth (optical density) and biomass (dry weight), nitrate uptake and medium pH were monitored every 2–4 days, pigments were assessed spectrophotometrically, and fatty acid methyl esters (FAME) were analysed by Gas Chromatography Coupled with Flame Ionisation Detector (GC-FID) after direct transmethylation of the algal-biomass. Results highlight the outstanding potential of two particular species: *Nannochloropsis oculata* for EPA production, and *Isochrysis galbana* for DHA production. Additional experiments were conducted to assess the effect of nitrate and phosphate concentrations, and the effect of photoperiod on their EPA/DHA contents and respective productivities. Further, strain improvement of *Nannochloropsis oculata* for EPA productivity was undertaken using natural selection performed by serial dilution under specific growth conditions. The goal is to obtain a naturally and genetically improved strain that is biologically adapted to the temperature in Ireland.

DOES POSITION MATTER? PHOTOSYNTHETIC PARAMETERS OF GRACILARIA BIRDIAE AND G. CAUDATA (GRACILARIALES, RHODOPHYTA) FROM NORTHEASTERN BRAZILIAN COAST

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Oscillating environmental conditions can cause directional evolutionary change, and light is one of the most variable factors in the environment. Distinct species could produce similar photosynthetic phenotypes when exposed to ecological conditions capable of influencing these processes. *Gracilaria birdiae* Plastino & E.C. Oliveira and *G. caudata* J. Agardh are important sources of agar production in Brazil. Although morphologically similar, previous inter crossing experiments, as well as reproductive structure analyses and recent molecular data have confirmed these as separate species. The aim of this study is to verify whether similar photosynthetic phenotypes could arise in morphologically alike and phylogenetically close alga species exposed to similar environmental conditions. The photosynthetic parameters were assessed from data collected in nature, with the use of *in vivo* chlorophyll *a* fluorescence technique. Two populations of *G. birdiae* and *G. caudata* were evaluated along the Brazilian coast: Rio Grande do Norte (RN) and Ceará (CE). In RN, where individuals of both species occupy distinct positions on the sandstone shore. Differences were found among most photosynthetic parameters: photosynthetic efficiency (αETR), effective quantum yield (ΔF/Fm') and light saturation (Ek). Nevertheless, in CE, where both species were found growing at a similar tidal level, constantly exposed to similar environmental conditions, no differences were found among all photosynthetic parameters (αETR, ΔF/Fm', Ek, and maximum electron transport rate ETR_{max}), regardless of the reproductive stage. Previous laboratory studies, done with individuals of *G. birdiae* and *G. caudata* from CE, have found that under control conditions both species present distinct photosynthetic characteristics. Our results suggest an environmentally-induced phenotype selection that could persist as long

as its inducing factor does, or until selection changes the threshold for its production. Defining the range of physiological variation within cryptic species complexes is essential for successful projections of future ecological changes.

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PHYLOGEOGRAPHIC PATTERNS OF GRACILARIA CAUDATA (GRACILARIALES, RHODOPHYTA) ALONG THE BRAZILIAN COAST

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The Brazilian coast extends for nearly 9,000 km and across 39 degrees of latitude, historical processes suggest regional variations seen nowadays in the genetic structure of several coastal species. *Gracilaria caudata* is a red alga whose geographical range extends from the Caribbean to the south of Brazil (27°S) and some evidence of genetic structuring among natural populations has been observed in previous work. The aims of this study are to assess the genetic connectivity among populations of *G. caudata* along the Brazilian coast and the effectiveness of putative biogeographical barriers on this connectivity using both cytoplasmic and nuclear markers. Seven populations were sampled along the Brazilian coast: Ceará (CE), Rio Grande do Norte (RN), Paraíba (PB), Pernambuco (PE), Bahia (BA), Espírito Santo (ES), and São Paulo (SP). Phylogeography analysis of the 313 sequences revealed the existence of 26 mitotypes

structured in three haplogroups corresponding to three geographic regions, with none of these haplotypes shared among regions. We thus revealed the occurrence of phylogeographic barriers separating the northeast localities of CE, RN, PB, and PE from the central locality of BA and the southeast localities of ES and SP. Likewise, the genetic structure analysis of the 411 tetrasporophytic (2n) individuals genotyped using 15 polymorphic nuclear microsatellite loci supported the existence of a differentiation between the northeast and a group composed of BA and the other southeast localities. The results from STRUCTURE within each of these two subgroups infer an optimal K of K = 3 and, in total, six genetic clusters were observed. The existence of barriers to gene flow such as the Vitória-Trindade seamount, a chain of submarine volcanic hills located between BA and PE, may account for the phylogeographic pattern observed in *G. caudata*. This is the first phylogeographic work with benthic macroalgae along the Brazilian coast.

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PRODUCTION OF NANOPARTICLES FROM MICROALGAE CELLULOSE: NANNOCHLOROPSIS GADITANA

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Nannochloropsis gaditana is a microalga belonging to the class of Eustigmatophyceae. This particular microalga is a well-studied species as it is rich in lipids and is used for biodiesel production. A low concentration of NaOH (2%) and sodium hypochlorite (6%) allowed us to obtain relatively pure cellulose from *N. gaditana*, which was analyzed by Fourier transform infrared (FT-IR) and Solid NMR. Nanoprecipitation, also known as solvent displacement was used to produce nanoparticles from this microalgal cellulose. Atomic force microscopic (AFM) images showed that the particles exhibited a spherical shape with sizes of $164 \pm 38 \times 171 \pm 21$ nm (height \times horizontal). This characteristic makes these nanoparticles an interesting candidate as an excipient in aerosols or ointments, as their size would allow penetration of the lung barrier and of the skin.

ACCESS TO ALGAE: LEGISLATION, SCIENCE AND ETHICS

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Both micro- and macroalgae are already widely commercialized and many products are already on the market. With their high levels of oils, carbohydrates, sugars and proteins, algae are used by humans for wide range of applications such as food, renewable fuel, cosmetics, neutraceuticals, pharmaceuticals and aquaculture application and billions of dollars are being invested in the research and development of algae-based technologies. This remarkable growth of algae-based industry in turn enhance marine bioprospecting activities, which have the potential to sustainably deliver considerable wealth and business opportunities to local economies. Various international conventions and protocols emphasize development of fair and equitable sharing of the benefits arising from the use of marine resources with the local communities and aim to secure supplier or user countries. This presentation will shed some light on the access and/or use of Macro-and Microalgae, in high sea or territorial waters, providing robust ethical context for further bioprospecting activities.

MICROALGAE BIO-PROSPECTING: AN OFTEN OVERLOOKED KEY TO SUCCESS IN THE DEVELOPMENT OF COMMERCIAL MICROALGAL TECHNOLOGIES

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Microalgae entrepreneurs often cite the incredible range of diversity and the large numbers of existing species to support their claims of the “vast potential” of microalgae for commercial exploitation. Relatively recent estimates of the number of algae species run from 30,000 to 1,000,000. Historically however, once these commercial ventures are launched, bio-prospecting often becomes limited to a simple exercise of looking through existing culture collections to find target strains for development. Since the majority of these culture collection strains were originally collected without a protocol emphasizing a commercial bio-prospecting focus, they almost always have several key performance limitations that ultimately prove to be difficult or

very expensive to overcome, eventually leading to failure of the ventures.

By utilizing a bio-rational collection and screening process focused on fermentation as the target production process, we were able to isolate a novel type and strain of microalgae (*Schizochytrium* spp.) for the commercial production of DHA. This technology has now been in continuous large-scale production for over 20 years yielding both DHA-rich biomass and oil, and still remains one of the best microbial technologies for producing these commercially important products. From both a cost of production and a patent protection perspective, I describe how critically important an emphasis on bio-prospecting ultimately proved to be for both the short-term and long-term commercial success of this production technology. I close by describing the essential steps in developing a bio-rational collection and screening process, and re-emphasize its role for successful microalgal technology development.

BURNING COAL SPOIL HEAPS AS A NEW HABITAT FOR THE EXTREMOPHILIC RED ALGA *GALDIERIA SULPHURARIA*

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Galdieria sulphuraria (Cyanidiales) is a worldwide acclaimed thermoacidophilic red microalga with a limited distribution due to special conditions required for growth and metabolism. Until now, the alga was almost exclusively restricted to acid geothermal environments around the world. However, we have found this species on the surface of a burning coal spoil heap in central Europe, the first record of *G. sulphuraria* in this type of habitat. A *rbcL* phylogeny confirmed that the population of this extremophile belongs to the continental European lineage and we consider Italian geothermal sites as a potential source of Czech *G. sulphuraria*. The dispersal of unicellular red microalgae is far from fully understood and the discovery of *Galdieria* in another region of Europe on a relatively newly established site allows us to understand better the distribution patterns and dispersal abilities of this ecologically important algal group. In addition, we have also analyzed the phylogenetic position of *Galdieria* strain CCALA 965 isolated seventeen years ago from a highly acidic site without geothermal activity in Czechia and have confirmed it

to belong to the species *G. phlegrea*, until now known only from Italy.

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CONTRIBUTIONS TO THE IDENTIFICATION OF BENTHIC AND FLOATING SARGASSUM (PHAEOPHYCEAE, FUCALES) FROM THE BRAZILIAN COAST

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Among brown algae from the Brazilian coast, *Sargassum* is the most important with respect to canopy cover in the shallow sublittoral zone. Benthic (9) and holopelagic (2) species of *Sargassum* are reported. They are classified into the subgenus *Sargassum* and two lower taxonomic categories: Acanthocarpicae and Malacocarpicae. The delimitation of those species is not an easy task because of the intraspecific variability and overlapping diagnostic characters such as observed between *S. hystrix* and *S. platycarpum*, *S. filipendula* and *S. vulgare*, *S. natans* and *S. fluitans*. Effective contributions for the subdivision of the genus into infrageneric taxonomic categories are available via molecular analyses, however no consensus for a molecular marker with resolution at the species level exists. Among different DNA barcodes evaluated for this subgenus, ITS-2 and RubisCO are inadequate, but mitochondrial markers show some potential. Recently developed primers that amplify *cox2* and *cox3* genes enabled the differentiation between forms of *S. natans* (I vs. VIII) and *S. fluitans* from the Western Atlantic, however it is unknown whether these primers can differentiate between benthic species. This study analysed the genetic differences among benthic and holopelagic *Sargassum* specimens from different sites along

the Brazilian coast, using *cox2* and *cox3* markers. Benthic specimens from the southeastern and northeastern coast and fragments stranded in beaches of Pará state were sampled and separated into five morphotypes. *S. natans* and *S. fluitans* were differentiated by *cox2* and *cox3*. *Cox2* also separated benthic samples from holopelagic ones. Except for one benthic sample from the northeastern coast, the other Brazilian samples grouped in one clade, supporting the division of the subgenus *Sargassum*. The stranded *Sargassum* species from Pará is closely related to *S. fluitans* and *S. natans* from Western Atlantic. We conclude that mitochondrial markers are useful for elucidating relationships within *Sargassum*.

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DOWN-REGULATION OF POLYUNSATURATED ALDEHYDES PRODUCTION BY SKELETONEMA COSTATUM UNDER HIGH CO₂ LEVELS

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Skeletonema costatum is one of the most widespread diatoms. Among its physiological characteristics, *S. costatum* is able to produce polyunsaturated aldehydes in response to stress conditions, especially during the end of blooms. Several results in the literature suggest that these molecules can act as allelochemicals to other plankton species and infochemicals that mediate plankton interactions. Ocean acidification is known to affect several aspects of diatom physiology and photosynthetic metabolism has been shown to be down-regulated under high CO₂ conditions for diatoms, decreasing the cellular pools as a response to enriched CO₂. Here we compared polyunsaturated aldehyde production of *S. costatum* in semi-continuous cultures acclimated for three weeks to actual CO₂ (380 ppmv, Low carbon, LC) and predictable future High CO₂ (1,000 ppmv, High Carbon, HC) under nutrient and light replete conditions. Our results showed that *S. costatum* produced two main aldehydes, 2E/4Z-Heptadienal (HD) and 2E/4Z-Octadienal (OD), and traces of 2E/4Z/7Z Octatrienal (OT). The potential production of total PUA was reduced from 36.28 ± 1.88 mM at LC to 10.35 ± 5 mM at HC and this trend

was also observed when normalized by cell (3.01 ± 0.77 fg cell⁻¹ to 1.11 ± 0.17 fg cell⁻¹). These results demonstrate that enriched CO₂ would reduce potential PUA production in *S. costatum* affecting its plankton interactions.

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TRICHORMUS VARIABILIS (NOSTOCALES, CYANOBACTERIA) EXOPOLYMERS FOR HYDROGEL APPLICATIONS IN ENZYME ENCAPSULATION AND TISSUE ENGINEERING

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Cyanobacterial Extracellular Polymeric Substances (EPS) are highly complex and variable substances exhibiting hydrophilic and anionic properties. EPS can comprise heteropolysaccharides of six or more sugar units, proteins, nucleic acids and lipids. EPS highly retentive properties make them promising for producing hydrogel scaffolds for drug delivery and tissue engineering. Released-EPS (R-EPS) were obtained by intensive indoor cultivation of a strain, VRUC 168, of the cyanobacterium *Trichormus variabilis* (Kützing ex Bornet & Flahault) Komárek & Anagnostidis, isolated from sediments of a Mediterranean coastal lagoon, in polyethylene bags (10 L). Biomass productivity reached 0.039 ± 0.001 g (d.w.) L⁻¹ d⁻¹. At stationary phase (day 20) the culture medium was collected and R-EPS concentrated by centrifugation and rotary evaporation. R-EPS were then precipitated in cold ethanol and the pellet freeze-dried with a yield of 2 g. The material was characterized by RP-HPLC, spectroscopic analyses, cytochemical staining, BCA and phenol-sulphuric acid assays, evidencing the presence of carbohydrates with sulfated and carboxylic groups

and 3.5% w/w of proteins. FT-IR spectrum showed peaks within 2,900 and 3450 cm⁻¹, attributable to -CH and -OH group vibrations. R-EPS were then used to produce hybrid hydrogels (EPS-Hys) which were stable over time and resistant to dehydration and spontaneous hydrolysis at 37°C. The mechanical properties of EPS-Hys were also assessed by rheological analysis. Additionally, EPS-Hys were tested for enzymatic encapsulation using thiosulfate:cyanide sulfur transferase (TST) enzyme. EPS-Hys with the embedded enzyme showed TST activity, which was preserved over time with only 14% decrease after overnight incubation at 37°C. Finally, EPS-Hys were used as 3D scaffolds for culturing human cardiac mesenchymal stem cells (cMSCs). An increase in cell viability of 80% was evaluated after 3 day growth suggesting a non-cytotoxic effect of the EPS-Hys. These preliminary data suggest potential applications of EPS-Hys for environmental biotechnology in the remediation of cyanide polluted aquatic ecosystems and for tissue engineering.

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STUDIES ON SPECIES DIVERSITY OF FRESHWATER ALGAE LOCATED IN KYIV UPLAND (UKRAINE)

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The algal flora of Ukraine is considered as having been studied in detail and is represented by nearly 5,200 species (ca. 6,300 infraspecific taxa), including the type specimens of some species. However, summary and comprehensive information about the central part of the Forest-Steppe zone – Kyiv Upland has been absent until now. The lack of consolidated data makes it difficult to compare the floristic composition with adjacent territories. The current paper presents the results of a preliminary floristic study of algal species diversity of the reservoirs at Kyiv Upland. Algal floristic studies for a period over 1.5 centuries were analyzed. According to the literature data, the species composition of the investigated area is made up of 641 species (669 infr. taxa) which represent 9 divisions and 252 genera. The taxonomic structure is characterized by a leading role for the Bacillariophyta, Chlorophyta and Cyanoprokaryota divisions. The material for the present work consists of algal samples collected in the different type of reservoirs (small rivers, lakes, fish-ponds, ephemeral ponds) for the period 2014–2016. Algae were studied using light microscopes, and a scanning electron microscope JSM-6060 LA (for studying the surface structure of the valves of diatoms).

As a result of this original research, we found 321 species (338 infr. taxa). The dominant complex of the investigated area consisted of widely distributed species – Tetraëdron minimum (A. Braun) Hansgirg, Desmodesmus communis (E. Hegewald) E. Hegewald, Coelastrum astroideum De Notaris, Crucigenia tetrapedia (Kirchner) Kuntze, Monoraphidium arcuatum (Korschikov) Hindák, Monoraphidium contortum (Thuret) Komárková-Legnerová, Pediastrum duplex Meyen, Pseudopediastrum boryanum (Turpin) E. Hegewald, Cocconeis placentula Ehrenberg and Melosira varians C. Agardh. Also revealed were more than 20 rare species including – *Achnanthes lauenburgiana* Hustedt, *Tribonema ulotrichoides* Pascher, *Acutodesmus javanensis* (Chodat) Tsarenko, *Radiococcus planktonicus* J.W.G. Lund, *Gloeotaenium loitlesbergerianum* Hansgirg and *Botryococcus terribilis* Komárek & Marvan. Among them were two species *Anabaenopsis circularis* (G.S. West) Woloszyńska & V. Miller and *Phacus tropidontus* Conrad which are new for Ukraine.

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IN SITU IDENTIFICATION AND LABORATORY STUDIES ON THE INTERACTIONS BETWEEN FILAMENTOUS BROWN ALGAL ENDOPHYTES AND KELPS

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In the marine environment, small endophytic filamentous algae are known to invade stipes and fronds of kelps, however little is known about the molecular basis of this interaction and the physiological impacts. In this context, we investigated the molecular diversity and host specificity of endophytic brown algae in wild kelp populations by sequencing the COI and ITS1 regions of more than 50 endophytic samples isolated from five different kelp species. The endophyte *Laminarionema elsbetiae* was isolated from *Saccharina latissima* and *S. japonica* tissues. In addition, we identified three species of the genus *Laminariocolax*, one of which is *L. acidioides*, an endophyte with a worldwide distribution and a broad range of hosts. The other two

identified species – *L. tomentosoides* and an as yet undescribed species of *Laminariocolax* – were associated with different kelp species of the North Atlantic sampling sites. Our results suggest that specific host-endophyte patterns exist, as found in kelps from Brittany, but they are variable with location as observed for other regions like Western Scotland and Helgoland.

To gain further insight into the physiology of host-endophyte specific interactions, we set up an experimental design focused on the endophyte *L. elsbetiae* with its main host *S. latissima* and the occasional host *Laminaria digitata*. A co-cultivation bioassay was developed to monitor the impact of the endophyte on growth of laboratory-grown kelp sporophytes. Whereas no effect was observed on *S. latissima* plantlets, growth of *L. digitata* significantly decreased in less than a week when co-cultured with *L. elsbetiae*. Preliminary results on the prevalence of endophytic filaments in the kelp tissue suggest that defence reactions against *L. elsbetiae* were triggered in *L. digitata*, but not in *S. latissima*. Further analyses of endophyte-induced responses in kelps will help to decipher host specificity and to better understand the basis of this interaction.

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INTERSPECIFIC COMPETITION BETWEEN ARCTIC KELPS ON DIFFERENT SPATIAL AND TEMPORAL SCALES

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The kelp species *Alaria esculenta* and *Laminaria digitata* are co-occurring in the shallow sublittoral at depths between 0 m and 8-10 m at our Arctic study site in Kongsfjorden, western Spitsbergen but they also co-occur at their southern distribution boundary in Brittany, France. The physical driving forces for their vertical and geographical distribution pattern are partially known but the effect of abiotic factors on interspecific competition has never been

investigated. The latter might become increasingly important, especially under future global warming scenarios.

In our study, we thus investigated the interaction of early recruitment stages (spores, gametophytes, juvenile sporophytes) between *A. esculenta* and *L. digitata* under varying temperature conditions on different spatial (μm to cm) and temporal scales (days to weeks), comparing responses of clonal and freshly derived gametophytes in laboratory experiments, in single culture controls and diverse co-cultivation set-ups.

Germination and development into sporophytes of both species were positively stimulated by a temperature increase from 5°C to 10°C . However, at 15°C the development of *A. esculenta* was strongly retarded and although some eggs were released from oogonia, no sporophytes developed. In general, sporophyte development and growth was much faster in *A. esculenta* than in *L. digitata* leading to out-competition of the latter species in long-term experiments at 5°C . During gametogenesis transient interspecific competition effects occurred, but always in favour of *A. esculenta*. The susceptibility towards both species interaction and temperature was species-specific and different developmental stages were affected differentially. All observed effects between the two kelp species may be interpreted as a consequence of resource competition and less probably, as consequence of allelochemical interaction. As temperature affected the outcome of competition, favouring *A. esculenta* at lower temperatures and *L. digitata* at higher temperatures, changes in community structure in the future are likely to occur.

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QPCR-BASED DETECTION OF FILAMENTOUS BROWN ALGAL ENDOPHYTES IN SACCHARINA LATISSIMA

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The marine brown macroalga *Saccharina latissima* is an important primary producer in temperate to cold northern hemisphere shores and an economically relevant seaweed with high industrial potential. Morphological changes – such as dark spots, twisted stipes and deformed blades – have been observed in wild populations and seaweed farms. The putative cause for the occurring symptoms is the filamentous endophytic brown alga *Laminarionema elsbetiae*, which is known to invade the stipes and fronds of its hosts. We conducted field surveys to measure the prevalence of *L. elsbetiae* in *S. latissima* populations and to understand its ecological impact. Until now, epidemiological studies on kelp endophytes were mainly based on the examination of microscopic sections, followed by time-consuming isolation and cultivation steps in order to identify the endophyte. As a novel approach, we developed and validated a highly specific q-PCR assay for relative quantification of the endophyte *L. elsbetiae* within its host *S. latissima*. We applied the assay to localize and quantify endophytic filaments of *L. elsbetiae* along *S. latissima* sporophytes and showed that these filaments are distributed unequally within the host, with significantly more endophytes being present in the blade tip than in other parts. Furthermore, an epidemiological study conducted over one year in Brittany showed that seasonality and environmental factors have a significant effect on both endophytic prevalence and infection rates, with higher relative infection rates during summer. Finally, our results obtained from laboratory-raised sporophytes that were transferred to a seaweed farm suggested that the infection might occur early in the sporophytic life stage of the kelp. Altogether, these qPCR-based studies allowed us to explore the dynamics of the endophytic infection during the *Saccharina* life cycle in field populations of this kelp. In the future, this approach could be applied to study other host-endophyte pairs using specific primers.

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SYSTEMATIC REASSESSMENT OF RIVULARIACEAE (NOSTOCALES, CYANOPHYCEAE)

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The classification system of cyanobacteria has changed considerably in recent years, with major taxonomic revisions, corrections in nomenclature, and proposals of new taxa. However, there are still groups whose position in the cyanobacterial system has not yet been satisfactorily defined. This is the case for the family Rivulariaceae, formed traditionally by the genera *Calothrix*, *Isactis*, *Rivularia*, *Dichothrix*, *Sacconema*, *Gardnerula* and *Gloeotrichia*. This family is characterized by having monoseriate filaments with a marked taper and a basal heterocyst for much of their growth cycle and by the development of terminal hairs at the apical ends. Previous studies have shown a high level of genetic diversity in the genus *Calothrix*. Heterogeneity has also been observed in *Rivularia* although complete revisions have not been undertaken. The genus *Gloeotrichia* still awaits a molecular characterization and only a few sequences are available, whereas the other genera have not been sequenced yet. In this study we investigated more than 80 tapering cyanobacterial strains together with environmental samples from different habitats. To research the relationships among these genera we analyzed morphological characteristics and performed phylogenetic analysis of several taxonomic markers (16S rRNA genes, *rbcLX*, *rpoC1*). Molecular analysis confirmed the separation of the genus *Gloeotrichia* into the new family Gloeotrichiaceae, belonging in the vicinity of Nostocaceae. Results also revealed that the majority of *Calothrix* species are clearly separated from *Rivularia*. In this way, we propose a new family, Calothricheae, to including several clades of *Calothrix*-like taxa together with the recently described genus *Macrochaete* and *Dichothrix*, whereas Rivulariaceae would be formed by the genera *Rivularia*, *Isactis*, *Sacconema* and *Gardnerula*.

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PIRATE: A PIPELINE TO RETRIEVE AND ANNOTATE TRANSPOSABLE ELEMENTS IN TISOCHRYSIS LUTEA GENOME

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Transposable Elements (TEs) are defined as DNA sequences able to move and spread within the genome of every organism. They are powerful forces for genetic change and play a notable role in the evolution of genomes. Once active, autonomous TEs can transpose by themselves and generate polymorphisms by new insertions/deletions. Potentially active under stressful events, these elements are recognized to promote genome adaptation through beneficial mutations. This role has been widely studied in some model species (e.g. human, terrestrial plants, insects), but little is known about microalgae. In a previous work, we underlined the putative involvement of active TEs in the genome adaptation of the haptophyte *Tisochrysis lutea*. However, because *T. lutea* is a non-model organism, only a draft genome assembly was available and its quality was not suitable to realize an accurate TE annotation. To achieve this, the reference genome of *T. lutea* was sequenced using the Pacific Bioscience and Illumina technologies. Then, a new bioinformatic pipeline named PiRATE was designed to identify and annotate TEs from genomic data of *T. lutea*. PiRATE is the first pipeline gathering all existing TE detection approaches from genomic data and its TE classification step has been optimized for microalgae genomes. Thus, PiRATE allowed us to estimate that around 4% of the genome of *T. lutea* is constituted of putative autonomous TEs. Subsequently, the mobility of TEs under stressful conditions will be investigated in order to estimate their impact on the genome adaptation of *T. lutea*.

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PEPPER DULSE: THE TRUFFLE OF THE SEA. INSIGHT IN OSMUNDEA PINNATIFIDA CULTIVATION

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Commercial seaweed production is “booming” with an estimated worldwide annual value of US\$ 6.4 billion. To meet market demands the sustainable production of algae for human food consumption/high value chemicals is of vital importance for the development of supply chains

for algal derived products. In Europe, there is a growing demand for seaweed for gourmet foods and low-volume high-value raw materials, primarily for use in the cosmetic sector. Currently in Scotland seaweeds are supplied mainly by natural harvesting. Yet, in the near future this will be insufficient to meet the rising demand and has the potential to lead to environmental damage. This project focuses on the supply of *Osmundea pinnatifida*, commonly called pepper dulse, a red macroalga currently collected from the wild and marketed dried as a peppery seasoning, due to its unique taste. It is mainly used in food preparation and a packet (5 g dry weight) retails at £12. The establishment of pepper dulse cultivation will provide a more consistent and sustainable product, reducing the environmental impact of harvesting wild material. The aim of this project is to improve crop yield and in addition gain an understanding of how to control the cultivation cycles to tailor the colour/texture/taste of the product according to the market's needs. The impact of cultivation conditions on the biochemical/chemical composition will be assessed, and the study involves scaling up of cultivation to a pilot scale, which will be fundamental for ensuring eventual commercial exploitation. In addition, the project is exploring the possibility of developing robust cryopreservation methods to allow a diverse range of materials to be held as genetic resources for subsequent research on conventional breeding and strain development. This challenging project represents the chance to translate research from the academic field into a commercial reality by researchers and industry working together.

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PHYTOPLANKTON RESPONSES TO RIVER OVERREGULATION – THE EXAMPLE OF THE SOUTHERN BUG RIVER (UKRAINE)

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The Southern Bug River belongs to the great rivers of the Black Sea basin, and is the greatest of those which the basin lies entirely within Ukraine. Its catchment area is approximately 63,700 km², with a river length of 806 km. The hydrological conditions of the river differ notably: there are rapids with outcrops formed by granite rocks of Ukrainian crystalline shield as well as plains segments and steady flow. Southern Bug is overregulated, with 187 artificial reservoirs

located along the river and its tributaries. In addition, human activities in the basin of the river are quite significant because of the South-Ukrainian energy complex, fisheries, housing and other sectors of the economy. Such anthropogenic activities have an impact on river nature. We studied phytoplankton responses to human activities on the riverbed. Algological, hydrobiological, and hydrochemical investigations were conducted on the Southern Bug River during the summers of 2007–2014. In total, 411 samples of plankton were collected from 41 sampling stations. A collected mass of published literature (1876–2004) allowed composition of a species list of algal plankton, and 318 species (337 infr. taxa) were revealed along the river. The original findings from the plankton samples in this study was 386 species (421 infr. taxa) of algae. The total amount of algae, recorded in plankton from the whole river from combined literature data and original findings is 501 species (563 infr. taxa).

The characterised material makes it possible to assume that a change in species composition has occurred over time. The dominant complex of taxonomic groups changed from diatoms to green algae. The predominance of green algae may be associated with a decrease in the flow velocity and greater regulation of the river when compared with the earlier period of the study.

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DIFFERENTIAL IMPACT OF SILVER STRESS ON CHRYSOPHYCEAE (STRAMENOPILES)

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Freshwater systems are highly endangered by anthropogenic introduction of xenobiotics, with silver ions being a prominent pollutant in recent years. Silver is increasingly used in industrial products and bactericidal applications. In aquatic environments, planktonic protists form the basis of food webs and are key organisms in terms of silver entering aquatic food chains. In order to understand and evaluate the effect of stressors on complex planktonic communities, a detailed investigation of the reactions of different trophic groups is required, regardless of the potential taxonomic bias. Chrysophyceae are particularly suited to address this issue as they are a closely related phylogenetic group comprising flagellates with contrasting nutritional strategies, including heterotrophic, mixotrophic, and phototrophic taxa. In a series of lab experiments, we tested the differential response of Chrysophyceae affiliated with different nutritional modes to silver stress and temperature increase. Regarding the nutritional mode of the organisms, we investigated to what extent food availability, light intensity and their combination have an effect on the stress tolerance. Our results showed, that heterotrophic strains were affected by silver, food concentration and temperature, where stressor combinations generally had an additive effect. The phototrophic strains were strongly affected by silver, whereas factors like light intensity and temperature had a minor effect on growth rates. The mixotrophic strains reacted differently depending on whether the nutritional mode was dominated by heterotrophy or by phototrophy.

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GREEN MONADS FROM LENINGRAD AND NOVGOROD REGIONS: ULTRASTRUCTURE CONTRIBUTION TO SPECIES IDENTIFICATION

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The revision of chlamydomonadacean flora of North-west Russia was launched by the study of green blooms. Fifty strains of green monad algae isolated from the Leningrad and Novgorod regions were referred to 7 species: *Chlamydomonas asymmetrica*, *C. debaryana*, *C. moewusii*, *C. nasuta*, *C. noctigama*, *Chloromonas typhlos*, *Microglena cf. opisthopyren*. All of them are new species records for regional flora. None of them was examined earlier as a bloom forming alga. *C. asymmetrica* and *C. debaryana* were the

most frequent. Species identification was carried out with light microscopy (LM) and transmission electron microscopy (TEM). High intraspecies variability (cell form and size, minor chloroplast characteristics, the number of pyrenoids, inclusions etc.) revealed by LM technique, did not let us identify most of them unequivocally, e.g. *C. asymmetrica* cells on agar media. In contrast, TEM research of different *C. asymmetrica* strains showed homogeneity of ultrastructural characters. Other analyzed species also had specific combinations of ultrastructural features (cell wall structure, organization of chloroplast and pyrenoid thylakoid system, stigma structure etc.). These data were in accordance with ultrastructural characters for every of 7 listed species, represented by different strains from world algal collections. Species identification was partially supported by 18S rRNA or ITS sequencing. The ultrastructural features for explored taxa proved to be the key moment in the green monad species identification. TEM characters are of great significance in verification of green monad phylogenetic trees.

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THE EVIDENCE FOR INCLUSION OF STEPHANOSPHAERA PLUVIALIS INTO LENINGRAD REGIONAL RED DATA BOOK

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Stephanosphaera pluvialis Cohn is considered a rare alga, an inhabitant of pools in rock, recorded from many different countries. Until 1 September 2013 it had never been seen in the Leningrad Region of Russia. The habitat is a 550 m 80 year-old granite dam on the western coast of Lake Ladoga (60°34 N, 30°42 E). The dam harbours a lot of technological holes in every granite block, so thousands of tiny puddles are available as potential habitats for *S. pluvialis*. Cells of *S. pluvialis* are organized as an 8-cell coenobium with a very remarkable morphology. They can be easily distinguished with an ordinary microscope. Once found in two stone holes filled with water, its coenobia was been detected

in different parts of the dam during four consecutive years. Until now, the Ladoga dam appeared to be the single place in the region where the alga was regularly observed. Beside the records from Ladoga dam and from two rock pools in the White Sea coastal region, there is no evidence of it being found after the mid 1930s in Russia. Therefore, *S. pluvialis* had been suggested for inclusion into the Red Data Book of Nature of the Leningrad Region. This work was partially supported by the Program “Living Nature” of the Presidium RAS and by grant of the President of the Russian Federation for the Leading Scientific Schools of Russia for 2016–2017, No. NSh 9513.2016.4.

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APPROACH APPLICATION OF BIOFLAVOR ALDEHYDE FROM KELP, SACCHARINA FOR ITS CRITERIA TAXONOMY

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The enzymatic aldehyde formation (aldehyde bioflavor) in *Saccharina angustata* especially, *n*-hexanal and 3*Z* and 2*E*-nonenal, could form through the lipoxygenase and hydroperoxide lyase pathway from arachidonic acid and linoleic acid through their fatty acid hydroperoxide. In this work, the other species of the genus *Saccharina* were investigated for their bioflavor formation and there was evidence that the species might generate such aldehyde as the same pathway as *S. angustata*. We studied enzymatic formation of *n*-hexanal and 3*Z* and 2*E*-nonenal in *S. japonica* in a variety of harvesting times and compared to *S. angustata* at the same harvesting time (the control) and variety of other species (*S. japonica* var. *religinosa*; var. *diabolica* and var. *longipedalis*), the comparisons were made with and without incubation using endogenous fatty acid substrate in all treatments. The volatile compounds were extracted by solid phase micro extraction to avoid pyrolysis formation and identified by GC/MS using internal standard method

for quantification. The increasing of those aldehydes was observed after incubation in all treatments comparable with the control and in all of the tested varieties. It was suggested that these compounds were the prominent compounds of this genus, *Saccharina* with the same pathway. This finding was proposed for alternative unique criteria of taxonomy for the genus by the investigation of the fatty acid aldehyde composition in kelp homogenate.

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BIOLOGICAL SOIL CRUST ALGAE OF THE POLAR REGIONS

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Biological soil crusts (BSCs) represent particular pioneer communities of various heterotrophic and autotrophic organisms associated with soil particles within and on top of the upper few millimeters of the soil. They have important ecological functions in primary production, nitrogen fixation, soil stabilization, water retention and biogeochemical cycles, and occur mostly in extreme, hot and cold semiarid/arid, nutrient-poor habitats. The main goal of this interdisciplinary project is a precise evaluation of the biodiversity of BSC microalgae isolated from the Arctic (Svalbard) and Antarctica (South Shetland Islands).

During the Svalbard expedition in August 2014 we found an unexpectedly high areal coverage of BSCs (up to 90%) and classified different BSC types. In addition, investigations on species composition revealed a very high biodiversity (100 species, Chlorophyta, Streptophyta, Ochrophyta), which was statistically significantly influenced by water availability.

The second field study was undertaken in January 2015 on Livingston Island, Antarctica. BSCs occupied up to 55% of the soil surface and similar BSC types were identified compared to Arctic BSCs. Samples from Ardley Island and King George Island hosted 106 algal species (Chlorophyta, Streptophyta, Ochrophyta including high proportion of

diatoms) and species composition seem to be controlled by micro-environmental conditions, such as substrate, topography and moisture. In addition, the effects of temperature and water stress on growth and photosynthetic tolerance widths of selected taxa were investigated in order to assess whether and how global change influences the ecological performance of BSCs and therefore, these data might enable better predictions of the development of vegetation in the Polar Regions.

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PHOTOSYNTHETIC PERFORMANCE OF THE RED ALGA *SOLIERIA PACIFICA* (SOLIERIACEAE) FROM TWO DIFFERENT DEPTHS IN THE SUBLITTORAL WATERS OF KAGOSHIMA, JAPAN

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Photosynthetic responses to PAR and temperature were examined in the red alga *Solieria pacifica* from deep (35 m) and shallow (5 m) portions of the sublittoral zone in Kagoshima, Japan. Dissolved oxygen sensors and pulse-amplitude modulated (PAM) fluorometry were used for the measurements of net photosynthesis, dark respiration rates, and photochemical efficiency. The photosynthetic performances of the deep- and shallow-water ecotypes provided substantial evidence of their adaptation to the ambient PAR and temperature conditions of their respective habitats. Both algal samples showed different temperature optima for photosynthesis: 19.5–19.9°C for *S. pacifica* from Mageshima (MAG), and 18.6–27.0°C for species from Yatsushiro Bay (YAT), that reflect the temperature ranges in the 35- and 5-m depths of the sublittoral zone, respectively. Maximum net photosynthetic rate ($P_{max} = 3.73 \mu\text{g O}_2 \text{ gfw}^{-1} \text{ min}^{-1}$), compensation PAR ($E_c = 21 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$), and saturation PAR ($E_k = 131 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$) were higher in YAT *S. pacifica* than in MAG samples ($P_{max} = 1.55 \mu\text{g O}_2 \text{ gfw}^{-1} \text{ min}^{-1}$, $E_c = 3 \mu\text{mol photons m}^{-2}$

s^{-1} ; $E_k = 15 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$), which can be attributed to the higher PAR levels experienced by the seaweeds occurring at depths of 5 m. The large depressions in effective quantum yields (Φ_{PSII}) after chronic PAR exposures, and failed recovery in maximum quantum yields (F_v/F_m) after dark acclimation of MAG *S. pacifica* suggest a greater tendency for photodamage in the deep-water ecotype. As for the shallow-water *S. pacifica* (YAT), complete recovery occurred only at 20°C. Low temperature limitation may account for the decreased gross photosynthetic rates and delayed recovery of post-dark acclimation F_v/F_m of YAT *S. pacifica* at temperatures below their optimum, which can occur during winter.

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PHYTOPLANKTON OF THE SOUTHERN OCEAN: PRELIMINARY DATA FROM THE 2016/17 ANTARCTIC CIRCUMNAVIGATION EXPEDITION

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The Southern Ocean refers to the region where Atlantic, Indian, and Pacific Ocean waters come together to encircle Antarctica. Despite the global importance of the Southern Ocean, knowledge of the controls on and interactions among the physical, chemical, and biological processes operating in Antarctic ecosystems are limited, largely because of a scarcity of *in situ* observational data. The Southern Ocean is becoming warmer, fresher, more acidic and less nutrient rich as a result of climate change. It is hypothesised that the phytoplankton will increase its productivity and that the community will change from large diatom cells to predominantly smaller celled species and flagellates, thereby reducing the amount of carbon sequestered into the deep ocean. The Antarctic Circumnavigation Expedition sampled all the bioregions of the Southern Ocean, including the Subantarctic Zone, Permanently Open Ocean Zone, Seasonal Sea Ice Zone, Marginal Ice Zone and the Antarctic Continental Shelf Zone, in a single summer cruise in 2016/2017. Through 103 underway and 26 CTD stations, a unique phytoplankton dataset was collected to establish a comprehensive baseline against which future

change could be measured. Preliminary results from selected stations will be presented.

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THE DIATOMS OF SHORE-PLATFORM EXTANT STROMATOLITE ECOSYSTEMS IN SOUTH AFRICA

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Extant marine stromatolites act as partial analogues of their Archaean counterparts, but are rare due to depleted ocean calcium carbonate levels and suppression by eukaryotic organisms. Unique, peritidal tufa stromatolites at the interface between marine and coastal groundwater seepage were discovered in South Africa in the past decade. Our aim was to investigate the benthic diatom community of these stromatolites to assess species richness, dominance and environmental drivers of succession over a period of one year. The stromatolite barrage pools consists of an upper pool that is freshwater dominated, a middle pool that varies between fresh and marine conditions and a bottom pool that is predominantly marine. Results indicate a relatively stable microalgal stromatolite community consisting of only five dominant species. Species richness increased to ~25 species in winter and was associated with marine overwash during storm events. The extreme conditions in the middle pool significantly reduced the species richness compared to the upper and lower pools. The main environmental drivers influencing the benthic diatom community were salinity and ocean swell. These results have implications in terms of interpreting community succession and differential layering in modern and fossilized stromatolites respectively.

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PHYTOPLANKTON SPECIES COMPOSITION CONTRIBUTING TO CARBON EXPORT – SEA TO SPACE PARTICLE INVESTIGATION

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Marine phytoplankton fuels the biological pump: the phyto-carbon can be naturally sequestered in the deep ocean through complex ecological and physical processes. When it comes to phytoplankton, it is not just the quantity, but also the carbon quality that matters, therefore species identification is crucial. During the Sea2Space cruise with R/V Falkor in January/February 2017, we investigated three stations in the North Pacific, ranging from the oligotrophic subtropical gyre to the upwelling-influenced coast of California. We collected seawater sampled with a CTD rosette in the mixed layer above the sediment trap platforms. Microscopical analyses revealed three distinct communities at stations congruent with their trophical status. At Station 1 microphytoplankton and nanophytoplankton reached abundances up to 4.8×10^3 cells L⁻¹ and 1.8×10^4 cells L⁻¹, respectively. The community was composed of 29 diatoms, 14 dinoflagellates, 10 coccolithophores and 4 crypto/silicoflagellate morphotypes. Dominant species were single-celled *Chaetoceros* sp., *Leptocylinndrus mediterraneus*, *Nitzschia bicapitata*, *N. braarudii*, *Gymnodinium* spp., *Gyrodinium* spp., *Calciosolenia murrayi*, *Ophiaster* sp. and *Michelsarsia elegans*. At Station 2, micro- and nano- abundances were up to 4.1×10^3 cells L⁻¹ and 2.2×10^4 cells L⁻¹ and the phyto-community was composed of 33 diatoms, 12 dinoflagellates, 11 coccolithophores and 5 hapto/crypto/silicoflagellate morphotypes. Dominant species were *Chaetoceros* sp., *L. mediterraneus*, *Nitzschia bicapitata*, *N. longissima*, *Gyrodinium* spp., *C. murrayi*, *C. brasiliensis* and *Discosphaera tubifera*. At the coastal Station 3 phytoplankton was the most diverse and the cell counts were one magnitude higher than at the other stations, with micro- and nanophytoplankton reaching up to 4.4×10^4 cells L⁻¹ and 1.1×10^5 cells L⁻¹. The community was composed of 57 diatoms, 15 dinoflagellates, 4 coccolithophores and 9 crypto/green flagellate morphotypes. Dominant species were *Chaetoceros convolutus*, *N. longissima*, *Pseudo-nitzschia*

CURRENT STATUS AND PROSPECTS OF PALEONTOLOGICAL RESEARCH ON CORALLINE ALGAE

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The record of potential ancestors of Corallinophycidae extends back to the Neoproterozoic with phosphatic fossils of complex pseudoparenchymatous thalli ca. 600 Myr old. The known first calcified pseudoparenchymatous thalli appear 120 Myr later, in the early Ordovician, and records of arguable reproductive structures in coralline-like fossils occur in the Silurian (430 Myr ago). There is a huge gap of about 300 Myr before the first appearance of coralline algae with reproductive structures similar to the ones in extant Corallinophycidae in the early Cretaceous (140 Myr ago). Since then, the coralline algal record is continuous and abundant. During the Cenozoic, corallines are rock-forming fossils, common in shallow-water marine deposits with little influx of terrigenous sediment. Many features used as diagnostic characters in morpho-anatomical taxonomy of living corallines can be identified in fossils, and for some years paleontological taxonomic practice did not substantially differ from botanical taxonomic procedures. Molecular phylogenetics, however, is profoundly challenging the morpho-anatomical taxonomic schemes of living corallines. Circumscriptions of subfamilies, genera, and species are being revised, sometimes with no diagnostic characters defining the new concepts of taxa. Paleontological research is especially affected by the lack of morphological definition of coralline taxa as only morphology is usually preserved in fossils. Calibration of phylogenetic models will probably focus on estimating the first occurrences of morphological traits of systematic significance at different levels and diverse ranges of time resolution. Paleocological inferences based on habitat requirements of living corallines will limit comparisons to species and genus groups. At the same time, paleocological models based on fossil coralline algal distribution in well-known ancient depositional systems should be developed to interpret coeval deposits in other localities. A pending task is to improve the knowledge of Precambrian to Jurassic coralline-like fossils in order to understand their diversity and potential relationships with extant Corallinophycidae.

A NEW PHASE IN THE SYSTEMATICS AND BIOGEOGRAPHY OF BANGIALES

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The Bangiales is an intriguing and challenging order of red algae with a cosmopolitan distribution and whose ancestors are now thought to date back to c. 1.6 billion years ago. Species within the order are highly diverse with superficially simple morphology but actually complex at the structural, life history and genetic level. Traditionally, the Bangiales contained two genera, *Bangia* (filamentous) and *Porphyra* (foliose/bladed), both with a shell-boring conchocelis phase. A generic revision of the order based on a two-gene phylogeny followed by phylogenetic studies at the regional level have led to the recognition of at least sixteen genera, seven filamentous and nine foliose. The largest genus is *Pyropia* (67 species), including several species of major economic importance in Japan, China and Korea. Taxonomic studies have also revealed a large number of undescribed species which points to the need to continue to explore understudied regions of the world if the extent of diversity is to be determined, species concepts defined and biogeographical boundaries drawn. Despite recent efforts, phylogenetic relationships are still unresolved. The application of DNA-based species delimitation methods has proved valuable in understanding relationships for the southeastern Pacific (Chile) and western Pacific (China) Bangiales. However, new approaches are required and the *Porphyra* genome and other high throughput sequencing are very significant. Here, I will review the taxonomy of the order, provide new data from the coast of China, discuss the possible origin of *Pyropia* and new genera, and the value of the biogeographical signal. I will also discuss possible ways to move forward with this group including consideration of discovery of the conchocelis phase in the field using a metagenomic approach. We are in a new phase in systematics and the Bangiales provides an excellent group with which to explore new different approaches and address many challenging questions.

YOUNG ISDR, A NEW INTERNATIONAL PLATFORM FOR EARLY-CAREER DIATOMISTS

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During the last International Diatom Symposium held in Quebec City (Canada) in 2016, the Young Diatomists section within the International Society of Diatom Research (ISDR) was launched. The idea behind this formation is (a) to address the interests of early career diatomists directly to the ISDR committee by an elected early career representative and (b) to assist early career researchers to build collaborations and develop skills needed for career enhancement. A better exchange with experienced diatomists should be facilitated providing workshops dealing with key topics of diatom research within future congresses such as the next International Diatom Symposium held in Berlin in 2018. The Young ISDR also tries to improve global networking with other international scientific organisations and related diatom research topics.

Here, we would like to present the results of a newly created socializing network of Young ISDR members including an interactive forum and a blog (<https://youngisdr.blogspot.com>), which both highlight job offers, possible funding, future workshops, research reports and key publications within the field of diatom research.

BIOASSESSMENT OF INTERMITTENT RIVERS AND EPHEMERAL STREAMS FROM THE MEDITERRANEAN BASIN USING DIATOMS: ARE CURRENT METHODS ADEQUATE?

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In Europe, the implementation of the Water Framework Directive has been unable to provide a solution to determine the ecological status of Intermittent Rivers and Ephemeral Streams (IRES). A lack of adequate methods (those developed are exclusively for perennial streams), hydrological data, and limited recognition in environmental policies have posed a major difficulty in their management. The TRIVERS project aims to provide new tools to improve the understanding of how IRES need to be assessed, preserved and managed in the Mediterranean region, where severe stream flow deficits are predicted facing global change. From April-December 2015 diatoms were sampled bi-monthly in 20 reaches located in Eastern Spain (including perennial, intermittent, and ephemeral streams) to assess responses of aquatic communities and biological indexes to flow intermittence. Rivers can be divided into permanent and intermittent. Permanent rivers always maintain water flow and can have hyperrheic (flooding) and eurheic (normal) water flows. Intermittent rivers are those that have a significant variation in flow regimes, either seasonal or random, and can lose their connectivity. The states intermittent rivers can display are eurheic, oligorheic (flow reduction), arheic (loss of connectivity, disconnected pools) and hyporheic (sediment highly saturated with water without pools). Results comparing intermittent and permanent rivers showed that at reaches where a trickle of water endured (oligorheic state), diatom sampling protocols were adequate. When flow ceased and disconnected pools were maintained (arheic state), the correlation between the four diatom indices used decreased significantly. After an arheic period, when the oligorheic state returned, they tended to decrease water quality significantly at the sites. On the other hand, the direct return of the connection in an eurheic state did not affect diatom index values significantly. Regarding community composition, the low flow volumes of oligorheic states, after washing over the substratum left by either arheic or hyporheic states, favoured the increase of subaerial diatoms.

EUGLENOID MOVEMENT: AN AVOIDANCE STRATEGY AGAINST ALGIVORES?

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Euglenoids have a unique pellicle composed of pellicular strips, which contributes to cell stability but also enables the characteristic euglenoid movement (metaboly). Some euglenoids, for example, certain *Euglena* species of the sub-group Serpentes (also known as “*Euglena deses* group”) are capable of extreme deformations of the cell, whereas other representatives are rigid and fast swimmers. During feeding experiments with algivorous vampyrellid amoebae (*Vampyrellida*, *Rhizaria*) we observed differential feeding success depending on the euglenoid species. The vampyrellid *Leptophrys vorax* consumed several species of *Euglena*, *Phacus*, *Monomorphina* and *Trachelomonas*, but displayed markedly reduced growth with *Euglena mutabilis* and *Euglena deses*. This inspired us to study the feeding strategy of *Leptophrys vorax* in more detail using time-lapse microscopy and to perform a quantitative feeding experiment with selected euglenoids. Rigid and metabolic euglenoids were offered to *Leptophrys vorax* in the living and dead condition, the latter eliminating any motility. The vampyrellid multiplied successfully with dead cells of all tested euglenoids and with live cells of *Phacus smulkowskianus* and *Euglena deses*. Live cells of *Euglena mutabilis* clearly prevented vampyrellid growth. As shown by detailed microscopy, *Leptophrys vorax* attempted to phagocytose *Euglena mutabilis*, but had severe problems ingesting the highly metabolic euglenoids. These data suggest that metaboly in certain phototrophic euglenoids might serve as an effective avoidance strategy against microbial predators.

OUTDOOR EXPERIMENTAL APPROACH FOR DETERMINING KELP PRODUCTIVITY: TESTING THE IMPORTANCE OF MACROCYSTIS PYRIFERA MORPHOLOGY IN ADULT SPOROPHYTES

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Size and morphology complexity matter when determining some physiological parameters in algae as large as the kelp, *Macrocystis pyrifera*. Having a total individual approach, we have developed a bioreactor experimental system that allows us to partially control environmental factors. Bioreactors are 400 L, semi-transparent fiber-glass tanks that can contain a whole adult sporophyte, therefore allowing us to perform semi-controlled experiments during a 14-day period without affecting the alga performance. Using this system, we carried out an experiment to determine photosynthesis (CO₂ uptake and C productivity) in sporophytes with differential allocation of biomass into the canopy and subcanopy (high C:SC ratio versus low C:SC ratio). Algae were collected in southern Chile (41°37'S; 73°04'W) and randomly assigned to the bioreactors (plus three control tanks with no alga). In each tank, we measured environmental parameters: pH, temperature, PAR light and alkalinity among others. CO₂ uptake were measured by an Infrared Gas Analyzer (IRGA) in a semi-closed system (air pumped into the tank at 20L m⁻¹; 500 µm bubble diameter; 200 ml min⁻¹ air entering the IRGA). Quantum efficiency, electron transport rate and the saturating irradiance for photosynthesis were also measured using a pulse amplitude fluorometer (PAM). The C:SC biomass ratio for the sporophyte used in the experiments average 1.4 in those with high ratio, whereas for those with low C:SC ratio the average was 0.6. Our results showed that plants with higher C:SC have a higher productivity when compared to those of low C:SC, with 21.9 and 11.6 µmol C s⁻¹ per kelp individual, respectively. When measuring photosynthesis in terms of CO₂ uptake we found no differences among plants with high and low C:SC (-75 mmol CO₂ min⁻¹ g⁻¹) which requires further interpretation in terms of methodology and plant physiology.

METABOLOMIC APPROACH TO SHED LIGHT ON THE METABOLIC PATHWAYS THAT REGULATE THE GROWTH AND MORPHOGENESIS OF ULVA MUTABILIS

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The green macroalga *Ulva mutabilis* Føyn interacts mutually with its microbial flora which influences growth, development, and morphogenesis. Together with two bacterial strains, *Roseovarius* sp. MS2 and *Maribacter* sp. MS6, the alga forms a tripartite community under laboratory conditions. Using this model system we have isolated an active small molecule from the supernatants of MS6 cultures (and also from large scale algal aquaculture) that can induce the effects of MS6 on *Ulva*'s morphogenesis. To investigate the molecular processes underlying the effects of MS6 in *Ulva* we have chosen the untargeted metabolomic approach. Axenic *Ulva* gametes were exposed, in triplicate, to the purified compound over the time course of 24 and 48 hours under standard *Ulva* growth conditions. Metabolites were extracted with an in-house protocol and analyzed using gas and liquid chromatography coupled to the high resolution Orbitrap mass spectrometer. We are currently correlating identified metabolites with changes that occurred at the genetic level when the axenic culture of *Ulva* was treated with the morphogenic compound. For that reason, RNAseq analysis of the same samples was conducted for transcriptomics. The integration of the two -omics techniques is a very promising approach that will shed light on metabolic pathways that are regulated during morphogenesis of *U. mutabilis*.

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THE GIANT KELP MACROCYSTIS PYRIFERA: EVIDENCE TO EXPECTED HIGH POTENTIAL FOR GENETIC IMPROVEMENT

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The giant kelp *Macrocystis pyrifera* is under strong and increasing exploitation pressure, mainly for alginates production and recently by the increasing demand for food/feed products, agrochemicals and bioenergy. In this context, there is an urgent need to move towards the development of sustainable aquaculture that incorporates genetic breeding programs. In this context, in the southern eastern-Pacific coast (SEP), advances have been made in the development of aquaculture of the giant kelp: pre-commercial seafarms

demonstrated that 124 wet ton ha⁻¹ yr⁻¹ of *M. pyrifera* can be achieved using wild individuals, establishing procedures for cultivation at hatchery and open sea. Also, bioethanol production technology was demonstrated at pilot scale under a biorefinery approach. Nonetheless, the actual challenge for giant kelp aquaculture involves the establishment of a breeding program for the domestication of the species. As a first approximation, the spatial structure of the genetic and phenotypic diversity of the giant kelp *M. pyrifera* was investigated using microsatellites markers to predict the population genetic structure present at the SEP, morphological analyses of wild sporophytes and crossing experiments were made to evaluate performance of F1 generation at hatchery and open sea cultivation. The results revealed three genetic clusters that coincide with the geographic distribution of the populations, however, lack of a pattern of isolation by distance suggest connectivity all along the SEP. Concordance between morphological and genetic clustering suggest the phenotypic variance has a strong genetic basis. In crossing experiments, no significant differences were observed between selfings and outbreeds, because of high intravariability observed. Higher genetic diversity than previously reported was revealed. *M. pyrifera* morphology variations along the SEP seem to have a genetic component besides the plastic variability that have been stressed previously, indicative of high potential for using this genetic variability for a sustainable aquaculture development. Results will be discussed in the context of the development of sustainable aquaculture.

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COMPARISON AMONG EPILITHIC DIATOM COMMUNITIES FROM AREAS OF INVASIVE CAULERPA SPECIES (CAULERPA TAXIFOLIA AND CAULERPA CYLINDRACEA) IN THE ADRIATIC SEA (NE MEDITERRANEAN)

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Diatom community structures at locations under the influence of different invasive *Caulerpa* species: *C. taxifolia* and *C. cylindracea*, were compared over a two-year interval. Samples for taxonomic study were taken from coastal rocks at three stations (island of Hvar, island of Mljet and the Dubrovnik area) in the eastern Adriatic Sea from November 2008 to October 2010. Light and electron microscopy examinations were used for qualitative analysis. Taxonomic analysis of diatom communities revealed 388 epilithic taxa belonging to 63 genera, with the most abundant being *Hyalosynedra*, *Rhopalodia*, *Navicula*, *Cocconeis*, *Berkeleya*, *Tabularia*, *Nitzschia* and *Grammatophora*. The study confirmed differences in species composition and abundance of epilithic diatoms among two *Caulerpa* sites. While the average number of taxa at the *C. taxifolia* sampling site (island of Hvar) was 77 per sample, the average number of taxa at the *C. cylindracea* sampling sites (Dubrovnik and Mljet) was lower at 64 per sample. The highest Shannon-Wiener Diversity Index values were during autumn and were almost the same for all three sampling sites, ranging from 5.26 to 5.34. MDS ordination revealed that location has more impact on diatom community structure than seasonal changes. ANOSIM performed on species abundance data confirmed that the difference between diatom communities among different sampling seasons was not significant. The results indicate that invasive seaweeds of the genus *Caulerpa*, which change the habitat by competing with autochthonous algae and diminishing the structural complexity, richness of species and diversity, evident at the macroscopic level have an impact on microflora as well. This study presents the first results on the taxonomic composition and distribution of epilithic diatoms from areas of invasive *Caulerpa* spp., and contributes to the broader and regional knowledge of diatom diversity in these challenged environments.

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USING SATELLITE REMOTE SENSING OF HARMFUL ALGAL BLOOMS (HABS) IN A COASTAL EUROPEAN SITE

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The impact of HABs (Harmful Algal Blooms) on public health and aquaculture has increased in the last decades and the necessity of their detection and monitoring is urgent. The use of satellite remote sensing for studying HABs is considered a promising technique to implement traditional in situ sampling for its advantages of large-scale, real-time, and long-term monitoring. The EC Horizon 2020 project Cyanolakes aims to develop a fully-automated processing system to provide information on the health risks from HABs and nutrient pollution for different European water bodies (lakes and coastal sites in Sweden, Romania and Italy). The service will exploit the wealth of information from the Copernicus Sentinel-3 and Sentinel-2 satellites to provide near real-time information and forecasts using innovative algorithms.

Among the study sites of the project, the Mar Piccolo of Taranto (Ionian Sea, Mediterranean) together with the northern Adriatic Sea hold up to 50% of Italian mussel production. HABs have been responsible for massive mussel kills since 1938, but in recent years the problem has worsened. Long-term research (1991–2014) has found evidence for the presence of about twenty-five harmful species. Among them twenty-three are potential producers of toxins (e.g. diarrhetic and amnesic shellfish poisoning). Besides the increase of the harmful eukaryotic microalgae, data have shown an increase in autotrophic picocyanobacteria, mainly represented by the genus *Synechococcus*, which can potentially produce microcystins, previously detected only in freshwater strains.

On the basis of in situ and remote sensing research, efforts will be made to build models and early-warning tools of use for authorities responsible for the safeguarding of public health, environmental protection and economic development of the coastal sites devoted to mariculture.

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A NON THERMOPHILIC, NON ACIDOPHILIC CYANIDIUM: THE LAST ENIGMA FROM SIBILLA CAVE

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The large volcanic area known as Campi Flegrei, situated northwest of Naples, Italy, includes both archaeological and volcanic sites of undeniable fascination. One of the most famous archaeological sites is the Sybil Cave, Cuma, where Sibyl prophesied, inebriated by the sulfur vapors coming from one of the forty volcanoes located in Campi Flegrei. What makes this site even more fascinating, along with its history, is that it preserves the most ancient life forms which are at the base of photosynthetic life. One of the most intriguing photosynthetic microorganisms is represented by *Cyanidium chilense* Schwabe (previously also known as cave Cyanidium); members of the genus *Cyanidium* have been frequently found in caves worldwide. Caves can be considered as extreme environments, where nutrient input, light intensity, temperature and humidity are limiting factors for microorganisms. In the present study we showed a morphological (light microscopy and CLSM) and ultrastructural (TEM) characterization of *C. chilense*. The study was also aimed at analyzing the microbial community living with *C. chilense* along with the physical-chemical interactions between microorganisms and substrate. The microbial community from Sybil cave was identified by molecular methods (16S and 18S rDNA, DGGE). Our results showed that *C. chilense* lived intermingled with bacteria, cyanobacteria and green unicellular algae colonizing the tuff cave walls of the Sybil Anthrum. SEM observations showed that the *C. chilense* community was embedded in a matrix that facilitated the establishment of strong bonds between biofilm and substrate. The microbial community was partly covered with crystallised microelements belonging to the substrate. Microanalyses of the microalgal biofilm revealed the typical mineralogical composition of the Neapolitan yellow tuff rock.

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GENOME SIZE VARIATION IN CHRYSOPHYTE GENUS SYNURA AND ITS ECOLOGICAL AND EVOLUTIONARY CONSEQUENCES

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Even though genome size has a substantial impact on both cell functioning and its size, the data on DNA content

in freshwater microalgae are almost non-existent. This strongly contrasts with the situation in higher plants and seaweeds. We present the first insights into the genome size variation in Chrysophyceae. Using flow cytometry, we analyzed DNA content in several representatives of the genus *Synura*, focusing mainly on *Synura petersenii* populations which have already shown substantial genome-size variation across Europe in our pilot analyses. The source of this diversity seems to be gradual increase/decrease in DNA content rather than genome doubling. Interestingly, this variation is not random but the genome size of *S. petersenii* individuals increases with latitude in Europe. Given that higher nuclear DNA content usually leads to an increase in cell size, our results are consistent with the so called “temperature-size rule” predicting increase in cell size with decreasing mean temperatures. Moreover, the genome size diversification may indicate independent evolution of lineages within *S. petersenii* and thus cryptic diversity.

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MARINE SEAWEEDS AS BIOLOGICAL INDICATORS TO ASSESS METAL POLLUTION IN A FUTURE NATURAL MARINE RESERVE (AGUELI ISLET, ALGERIA)

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Agueli islet is considered, at the local, regional and international level, a marine area of high water quality and biological, ecological and socioeconomic interest. It is being considered for a natural marine reserve. This natural heritage is now the subject of special attention because of the increasing urban and industrial waste being dumped untreated into Lake Reghaïa. The present work is a first in this area, and shows the accumulation of heavy metals in the tissues of macroalgae that are widely used as bioindicators of metal contamination.

The concentrations of Cr, Cu, Mg, Pb, Zn and Cd were measured in the tissues of three different marine macroalgae: *Ulva rigida* C.Agardh, *Enteromorpha compressa* (Linnaeus) Nees and *Ellisolandia elongata* (J.Ellis & Solander) K.R.Hind & G.W.Saunders. The algal samples were collected in the Reghaïa, Algiers region, at two stations: the first

a reference site and the second a polluted site characterized by discharges of waste and industrial water. The heavy metal concentrations showed significant variation depending on the sampling station. The concentrations are relatively high compared to other, similar studies. In general, *Enteromorpha compressa* was the strongest accumulator of Cd, Cr, Pb and Zn, in that order. *Ulva rigida* was the highest Cu and Mg accumulator. The accumulation of heavy metals follows the following order: Mg > Zn > Cu > Pb > Cr > Cd.

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NEW EDIBLE RED MACROALGA, *CALOGLOSSA BECCARII* DETONI FROM THAILAND

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The freshwater red alga, *Caloglossa beccarii* DeToni, consists of a dark brown filament and is attached to rocks in shallow streams located in various places in Thailand, especially in the southern part. However, no pharmacological studies have been carried to investigate the toxicity for local human consumers.

Therefore, the objectives of the study were to examine acute toxicity (AT), nutritional value, antioxidant activity and phytochemicals for this potentially edible alga. AT was tested with a single oral administration of the extract at a dose of 2 and 5 g kg⁻¹ body weight. Mortality, behaviour, amount of food intake, body weight, and any abnormalities of the visceral organs, were observed. The extract caused neither mortality, nor abnormalities. From proximate analysis, the alga was a rich source of minerals (calcium, potassium, manganese, iron and magnesium and selenium), carbohydrate (fiber and polysaccharide) and protein (arginine and lysine). It also provided a good source of vitamin C and linolenic acid. Ethyl acetate, ethanol, methanol and water extracts of the alga were screened for antioxidation activities using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) free radical scavenging. The highest TPC, DPPH and ABTS activity were in aqueous (20.868 ± 0.68 mg GAE g⁻¹), methanolic (IC50

= 0.086 ± 0.01 mg mL⁻¹) and ethanolic extracts (IC50 = 0.178 ± 0.01 mg mL⁻¹), respectively. It was also shown as a good source of phycocyanin and phycoerythrin. All results indicated the algae as safe, with useful properties.

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THE COST ACTION PHYCOMORPH: GETTING MORE KNOWLEDGE ON MACROALGAL DEVELOPMENT AND REPRODUCTION TO IMPROVE SEAWEED AQUACULTURE

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FA1406 "PHYCOMORPH"

PHYCOMORPH (COST Action FA1406; 2015–2019) is an international academic network comprising research teams dedicated to the identification of the biological events governing the development and reproduction of macroalgae. Despite the fact that macroalgae were amongst the first multicellular eukaryotes to emerge on earth, almost nothing is known about the molecular and cellular mechanisms involved in their development.

PHYCOMORPH contributes to fill the gap between past knowledge – mainly descriptive – and the increasing demand for answers to functional issues, by supporting networking activities involving 20 European countries and 8 non-European countries, and disseminating knowledge to the scientific communities and to the public.

For more about the activities and membership of Phycomorph, visit <http://www.phycomorph.org> and http://www.cost.eu/COST_Actions/fa/FA1406.

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BIOPHYSICAL MODELING OF TIP GROWTH IN THE FILAMENTOUS BROWN ALGA ECTOCARPUS

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Ectocarpus is a filamentous brown alga in which development is initiated by tip growth, a cellular process encountered in fungal hyphae and land plant pollen tubes and root hairs. We used a viscoplastic biophysical model (initially developed for root hairs) to numerically simulate tip growth in this alga. The model integrates several biological parameters, such as the internal cell pressure, the cell shape and the isotropic organisation of the cell wall. Based on the measurements carried out in situ on living algae, numerical simulations showed major differences in the strategies developed by this alga compared to the other tip-growing organisms.

DNA-ASSISTED BARCODING OF SEAWEED COMMUNITY IN TAIPING ISLAND IN SOUTH CHINA SEA

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The seaweed community of Taiping Island (Itu Aba Island) in the South China Sea was surveyed on 11–14 May 2017. A total of 66 species including 26 Chlorophyta, 10 Phaeophyta and 30 Rhodophyta were recorded from 12 sampling sites around the island. Six of these were located in the inner reef zone (before the reef crest) vertical to the coastline (0–2 m depth) and the other six were located in the coral reef zone parallel to the coastline at about 8 m depth. The common species were *Caulerpa* spp., *Dictyota* spp., *Galaxaura* spp. and *Halimeda* spp. in the inner reef zone. In the coral reef zone, algae coverage was under 5% and most of them were CCA. This study is the first survey of seaweed diversity with comprehensive DNA-barcoding

data in Taiping Island. Compared to previous studies, there are ten species newly recorded in this area. Our data will provide more insight into the seaweed biodiversity in the South China Sea.

GENOME ANALYSIS OF APOPHLAEA LYALLII (FLORIDEOPHYCEAE, RHODOPHYTA) PROVIDE INSIGHTS INTO THE ALGAL-FUNGAL SYMBIOTIC RELATIONSHIP

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Over a billion years of evolutionary history, red algae have adapted to a wide range of environments and diversified into more than 7,000 species. During this dynamic evolutionary process, some species have established parasitic or symbiotic relationships with other organisms which have contributed to their survival. One well-known example is the genus *Apophlaea*, a red algal genus of which is distributed only in intertidal zones of New Zealand. *Apophlaea* forms an obligate symbiosis with one marine fungal species *Mycosphaerella* sp. (Capnodiales). They are highly resistant to various stresses including ultraviolet radiation, salinity, and desiccation. To investigate these features with a genomic perspective, we have established the draft genome of *Apophlaea lyallii* (total size: 42.9 Mbp; GC contents: 42.6%; mean coverage: 164.48) using Pacbio and Illumina hybrid sequencing methods. Based on a genomic analysis, we describe the gene contents of the nuclear genome (number of genes: 14,350; BUSCO: 82.75%) and address the algal-fungal symbiotic relationship in relation to environmental tolerance.

FUCOSYLTRANSFERASE-LIKE PROTEIN, PTFUT FROM MARINE RED ALGAE PYROPIA TENERA (RHODOPHYTA) CONFERS OSMOTIC STRESS TOLERANCE

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Fucosyltransferases catalyze the transfer of an L-fucose to a protein or another carbohydrate that is used as a component of the cell wall and extracellular matrix in plants. However, the information on fucosyltransferase in the red algae is unclear. *Pyropia tenera* (Kjellman) are marine red algae that grow in the intertidal zone and lose more than 90% water during low tides every day. Based on the desiccation transcriptome from the gametophyte of *P. tenera*, we identified genes which respond to desiccation stress. From those, we cloned a gene that encodes a polypeptide of 591 amino acid residues similar to fucosyltransferase, containing the conserved amino acid residues for GDP-fucose binding site, and named it PtFut. Phylogenetic analysis showed that fucosyltransferases from red algae are evolutionarily much further distant from green plants than those of bacteria. PtFut showed upregulation of transcription in response to ABA treatment as well as desiccation and osmotic stress induced by mannitol. When chimeric PtFut-GFP was introduced into tobacco protoplast, fluorescence of the PtFut-GFP fusion proteins was observed predominantly in the nucleus. When PtFut was over-expressed in *Chlamydomonas*, the PtFut improved the growth of the transgenic cell under osmotic stress. These results suggest that PtFut may play a role in tolerance to desiccation stress, and provide insight into the understanding of the molecular mechanisms in growth of marine red algae in the intertidal zone.

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PLASTID INHERITANCE AND GENOME ARCHITECTURE IN THE ISOGAMOUS AND ANISOGAMOUS BROWN ALGAE

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The brown algae (Phaeophyceae) are photosynthetic Stramenopiles that all share a red algal-derived plastid via a secondary endosymbiotic event. In the different lineages of the brown algae, gametes exhibit a variety of morphologies during sexual reproduction. Gametes can be isogamous, anisogamous or oogamous. Anisogamous and oogamous species exhibit maternal inheritance of the plastid whereas isogamous species exhibit biparental inheritance of the plastid. A majority of the brown algae exhibit anisogamous or oogamous gametes and therefore their plastid is inherited from the maternal gamete. The order Ectocarpales represents an interesting exception, having isogamous gametes and therefore plastids transmitted biparentally to the next generation. To investigate the effect of biparental inheritance on the plastid genome features, we newly characterized the complete plastid genomes of three species from the order Ectocarpales: *Scytosiphon lomentaria*, *Petalonia binghamiae* and *Cladosiphon okamuranus*. Comparison between the plastid genomes of oogamous and anisogamous brown algae revealed that plastid genomes within the Ectocarpales had higher structural variation and rearrangements. We will discuss the evolutionary history of plastid genomes within the Ectocarpales and how organelle genome inheritance impacts the structural variations of organelle genomes in the brown algae.

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INCREASED LIPID PRODUCTION AND ALTERED LIPID COMPOSITION IN MICROALGA NEOCHLORIS OLEOABUNDANS BY OVEREXPRESSION OF DIACYLGLYCEROL ACYLTRANSFERASE 2

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Biodiesel production from microalgae is technically feasible, but not yet economically viable. Increasing microalgal

lipid triacylglycerol (TAG) content via genetic engineering could be a potential approach to improve microalgae as an economically viable biodiesel feedstock. Because genetic manipulation of microalgae requires the accessibility to stable nuclear transformation, we have successfully developed a stable nuclear transformation system of the oleaginous microalga *Neochloris oleoabundans* using electroporation. The cDNA encoding the key enzyme of TAG biosynthesis pathway, diacylglycerol acyltransferase (NeoDGAT2), of *N. oleoabundans* has been cloned and characterized. In this study, in order to increase TAG accumulation, *NeoDGAT2* expression cassettes was transformed into *N. oleoabundans*. The neutral lipid accumulation in the transformant, detected by Nile red staining, was accelerated and 1.9-fold higher than in wild type. The lipid bodies in the transformant visualized under fluorescence microscope were also larger. The *NeoDGAT2* transcript was 2-fold higher in the transformant than wild type. Remarkably higher TAG accumulation was found in the transformant than wild type: TAG content increased 1.8- to 3.2-fold up to $46.1 \pm 1.6\%$ dry cell weight and TAG productivity increased 1.6- to 4.3-fold up to 8.9 ± 1.3 mg/L/day, depending on cell culture condition. A significantly altered fatty acid composition was detected in the transformant compared to wild type: the levels of saturated fatty acid C16:0 doubled to 49%, whereas C18:0 was reduced triple to 6%. Long-term stability was observed in the transformants continuously maintained in solid medium over 100 generations in a period of about 4 years. Increasing TAG content in *N. oleoabundans* by over-expression of *NeoDGAT2* may offer the first step towards making microalgae an economically feasible source for biodiesel production.

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POTENTIAL USE OF THE POLYEXTREMOPHILIC MICROALGA GALDIERIA SULPHURARIA (CYANIDIOPHYCEAE, RHODOPHYTA) IN BIO-RECOVERY OF RARE METALS

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Galdieria sulphuraria belongs to Cyanidiophyceae (Rhodophyta), a group of red unicellular algae perfectly adapted to the environmental extremes found in volcanic and post volcanic areas, where temperatures rise above 50°C, and high sulphuric acid concentrations, generated by the oxidation of sulphur gaseous emissions, greatly reduce the pH to values prohibitive for the majority of eukaryotic life forms. The presence of a variety of metal transporters and different plasma membrane uptake systems gives the *G. sulphuraria* cell the ability to cope with toxic metals, which are commonly found in volcanic areas and acid mine, and neutralize biohazardous metals, such as arsenic, making it promising in biotechnological applications. The European Parliament's Committee on Industry, Research and Energy has requested a research study on the recovery of rare earths from electronic wastes in order to identify and analyse the potential of innovative technologies for this purpose. In order to develop an innovative and eco-sustainable process for the bio-recovery of rare metals from waste, we have assessed the impact cerium, dysprosium, neodymium and europium have on the growth, viability and ROS production in *G. sulphuraria*. The efficiency of adsorption and bioaccumulation was also assessed by mass spectrometry. We also propose the production of thin photobioreactors for outdoor photo-cultivation of microalgae using waste liquids from industrial processes.

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UNINVITED GUESTS? EFFECT OF BACTERIA ON GROWTH AND MATING OF THE BENTHIC DIATOM SEMINAVIS ROBUSTA

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Diatoms are important microalgae that shape biofilm communities. Signal molecules mediate their life cycle and mating, but only recently the first diatom pheromone was identified in the benthic diatom *Seminavis robusta*. The proline derived diketopiperazine (diproline) drives the chemoattraction of the mating partners. Since such a pheromone-based communication is a potential target for competing or pathogenic organisms, we asked if such

co-occurring species might interfere with the diatoms' chemical communication. We studied the pheromone chemistry and behavioural response of *S. robusta* both in the presence and absence of bacteria. Bioassays with different naturally co-occurring bacterial strains, quantitative target analysis to check the production and degradation of diproline, and an untargeted metabolomics approach were used to comprehensively characterize the interaction. We found that different bacteria have different effects on the mating and the growth of *S. robusta*, as well as on the prevalence of diproline, which is always higher in axenic conditions. Comparative untargeted metabolomics allowed us to survey the diatom chemistry, where amino acids and fatty acids were regulated in presence of bacteria. These could serve as nutrients for the bacteria.

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EXPLORATION OF THE BIOACTIVE LIPIDS DIVERSITY OF THE MARINE BENTHIC DIATOMS OF THE NANTES CULTURE COLLECTION (NCC)

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Marine diatoms can store high amounts of lipids and grow quickly. Unfortunately, they are little-studied and underexploited resources. The current work is part of the third axis "Biorefinery and Processes" of the Atlantic Microalgae (AMI) project with the global objective to promote the microalgae strains from the Nantes Culture Collection (NCC). This collection currently hosts more than 300 strains; mostly benthic diatoms with a tremendous unknown chemodiversity. In this work, the biochemical diversity of benthic diatoms strains from the NCC is studied. The main objective is to find out if the NCC diatoms strains are able to produce molecules of economic interest. To achieve this main objective, the implementation of a rapid chemotyping approach of the NCC strains molecules have been developed. Initially a bibliographic inventory of current and past knowledge of the main genus and species hosted in the NCC was carried out. It has been particularly focused on the families of molecules with high added value of pharmacological interest, health, nutrition,

and cosmetics, such as polyunsaturated fatty acids (PUFA) that could be biosynthesized under certain culture conditions. Results from this bibliographic investigation show that NCC strains can biosynthesize high value molecules. Based on bibliographic data, 90 strains assumed to produce PUFA have been selected from the NCC for analyses. The growth capacity of the strains has been analyzed using Water Pulse Amplitude Modulated based approaches to identified the day of the late exponential phase. Molecular profiles of the best growing strains were elucidated with the Fourier-transform infrared spectroscopy highthroughput screening system. Then for the most promising ones (ie., with a high lipid FTIR relative content) molecular structures of original fatty acids were elucidated using a mass spectrometry approach. The first results show that *Entomoneis paludosa* NCC18.2 contains an interesting fatty acid, the ecosapentaenoic acid (EPA), which possesses multiple health benefits such as anti-inflammatory, antihypertensive effects. Finally, potentially interesting strains for industrial exploitation will be tested in EOSS photobioreactors to optimize the biomass production.

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DIATOM-BASED BIOREFINERIES: EXOPOLYMERS, LIPIDS, AND BIOSILICA FROM PHAEODACTYLUM TRICORNUTUM, STAUROSIRELLA PINNATA AND THALASSIOSIRA WEISSFLOGII

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Diatoms are a versatile biomass feedstock upon which green biorefineries can be established. Extraction of valuable co-products from the same biomass shows application potential, reduces pipeline costs and is a prospect for novel development in biomedical and materials sciences.

In this work, a native isolate, VRUC 290 strain, of the colonial, araphid diatom *Staurosirella pinnata* (Ehrenberg) D.M. Williams & Round, from biofilms of a Mediterranean coastal lagoon, and two commercial strains, SAG 1090-1b and 122.79, of the marine species *Phaeodactylum tricornutum* Bohlin and *Thalassiosira weissflogii* (Grunow) G. Fryxell & Hasle, respectively, were mass cultivated in batch cultures in low cost polyethylene bags (10 L) indoors.

P. tricornutum reached the stationary phase at day 10, *S. pinnata* and *T. weissflogii* at days 14 and 12, respectively. *P. tricornutum* also showed the highest production with 0.29 ± 0.017 g DW L⁻¹, while 0.22 ± 0.0013 and 0.263 ± 0.005 g DW L⁻¹ were recorded for *S. pinnata* and *T. weissflogii*. No stress/optimized condition was applied in order to preserve biosilica pore integrity and homogeneity. The biomass of each culture, at the stationary phase, was dewatered by settling and centrifuging, and the exopolymers (EPS, 3 different operational fractions), cellular lipids and frustule biosilica were sequentially extracted by means of compatible reagents. Yield estimates and multidisciplinary characterization (light/electron microscopy, gas-chromatography, spectroscopy analyses) of extracts then followed, to highlight their potential application in different biotechnological fields. EPS showed high retentive properties suitable for application in hydrogel fabrication as a scaffold for tissue engineering. *P. tricornutum* had the highest lipid content 5.54% w/w: long chain polyunsaturated fatty acids omega-3 C20:5 (Eicosapentaenoic acid, EPA) and omega-6 C20:4 (Eicosatetraenoic acid) accounted for 26%, suggesting potential use in food ingredients and the nutraceutical field. *T. weissflogii* showed the highest nanostructured biosilica content, 2.93% and no ultrastructural anomalies were recorded in the frustules in SEM, suggesting suitability for photonics and dye trapping applications.

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TOWARDS THE BIOGEOGRAPHY OF MARINE BENTHIC DIATOMS ALONG THE COAST OF SOUTH AFRICA

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Marine botanical biogeography has been extensively documented in South Africa with a specific focus on macroalgae (seaweed). However, the knowledge of patterns in the geographic distribution of diatom flora is limited and the diversity of marine benthic diatoms along the coast of South Africa remains unknown. Temperature is a known determinant of biogeographical patterns and due to changing ocean temperatures and increased anthropogenic impacts, there is a degree of change with respect to species distribution in the marine ecosystem. The aim of the research is

to determine the biogeography of marine littoral diatoms along the coast of South Africa by determining the main environmental drivers responsible for such geographic patterns. Preliminary results will indicate the temperature and nutrient dynamics along selected sites and the species richness and diversity. Sampling will cover three biogeographical regions namely: cold- temperate, warm-temperate and subtropical. We will test the hypothesis that the microphytobenthos follows the same biogeographical patterns and breaks as macroalgae.

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GLOBAL INITIATIVE FOR SAFEGUARDING THE FUTURE OF THE SEAWEED INDUSTRY IN DEVELOPING COUNTRIES

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The seaweed industry is the fastest-growing of all aquaculture sectors, with an annual growth rate of 10% and a value in excess of \$5bn. 95% of this cultivation is undertaken by developing countries. Worldwide, seaweed farming provides income to millions of families in rural coastal communities and has enabled women to become economically active, in areas where fewer opportunities exist. Yet outbreaks of seaweed disease and pest infestations are threatening this industry, leading to dramatic (>15%) declines in yield, notably in three major seaweed-producing DAC-list countries, the Philippines, Tanzania and Indonesia, and is having catastrophic socio-economic impacts on the communities reliant on seaweed production. In the Philippines alone, losses over US\$ 100 million a year were attributed to disease, representing 15% of the country's farmed seaweed production, with similar reductions in production seen in Tanzania and Indonesia.

Key ecological and socio-economic challenges preventing the sustainable economic growth of this industry were

assessed and presented in a recent United Nations University Policy Brief and the UN's 'First Global Integrated Marine Assessment'. Two major challenges highlighted in these reports were the high vulnerability of crops to disease outbreaks and pest infestation and the lack of biosecurity measures and legislation governing the movement of seaweeds between regions and continents. Red seaweeds, in particular (*Kappaphycus* and allies), are an iconic example of how one group of seaweeds have been introduced to over 30, predominantly DAC-listed countries worldwide with minimal biosecurity measures in place.

RCUK GlobalSeaweed* is a new programme aimed at addressing the acute problem of disease and pest infestations by providing solutions, training and guidelines. It will also launch a GlobalSeaweed* Fund to support novel research opportunity and provide travel bursaries for researchers who can assist us with promoting the sustainable growth of this vital industry in seaweed-producing DAC-list countries.

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ULVA OHNOI (ULVALES, CHLOROPHYTA) AS A BIOFILTER IN IMTA-RAS SYSTEMS: INFLUENCE OF NITROGEN AND PHOSPHOROUS LEVELS IN ITS DEVELOPMENT

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This work was conducted in the frame of the IntegRAS project (AGL2013-41868-R), funded by the Spanish government, with the overall objective of optimizing the design and management of a sole-*Ulva* culture in an Integrated Multitrophic in Recirculating Aquaculture System

(IMTA-RAS) minimizing energy consumption, seawater renovation rate and improving the productivity and health of the system. In these type of systems, there are often high levels of dissolved inorganic nutrients, especially nitrate and phosphate. The present study aims to gain knowledge about the influence of the concentrations of these nutrients and their possible interactions in the development of *Ulva ohnoi*, the selected species in this project. The results show that at the levels studied there is a negative linear relationship in the growth of *U. ohnoi* with the increase of the phosphate concentration and a parabolic function of this growth in relation to the nitrate concentration. On the other hand, optimum concentrations of nitrate modulate phosphate toxicity to a small extent. Given the wide range of conditions tested here, the results obtained also allow us to conclude that the N:P ratio probably will not be a limiting factor for the cultivation of this species in IMTA-RAS systems. On the other hand, it is particularly interesting to note that under high levels of both nutrients the *Ulva* blade loses its consistency and fragments. These fragments are able to grow again at high rates in low-enriched media, so it could be an efficient vegetative propagation strategy.

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MORPHOLOGICAL AND MOLECULAR CHARACTERISTICS OF ULVA FLEXUOSA SUBSP. PILIFERA (ULVALES, CHLOROPHYTA) FROM MONTENEGRO

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Ulva Linnaeus (Linnaeus 1753) is one of the best known marine green algal genera which is responsible for widespread "green tides" and marine fouling, both of which are major global environmental problems. However, *Ulva* species are common throughout the world in marine and estuarine habitats and they are difficult to identify from morphology as a result of extensive variability of morphological traits and also a high intraspecific variation and

phenotypic plasticity, due to salinity variations and/or seasonal changes. As a result of field surveys in May 2015, we present four new locations for *Ulva* species in freshwater and marine habitats in Montenegro in the southwestern part of the Balkan Peninsula. Bright green *Ulva* thalli were found in a river, a ditch, the Milet Canal and on the rocky shore of the Adriatic Sea. *Ulva* specimens were identified as *Ulva flexuosa* subsp. *pilifera* (Kützinger) M.J. Wynne, using morphological and molecular analysis. X-ray fluorescence methods (TXRF and WDXRF) determined the following elements in the *Ulva* thalli: Al, As, Ba, Br, Ca, Cl, Cr, Cu, Fe, Hf, I, K, Mg, Mn, Na, Ni, P, Pb, Rb, S, Si, Sr, Ti, V and Zn. Montenegro has diversity in geology, climate, water biotopes and geography and this makes it a hotspot of biodiversity within Europe.

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FIRST RECORDS OF THE DINOFLAGELLATE GENUS DICROERISMA IN THE ADRIATIC SEA

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Several occurrences of the planktonic dinoflagellate *Dicrakerisma psilonereia* (Gymnodiniales) were recorded during a long term study in South Adriatic open waters from 2011 to 2017. This taxon was recorded in four cases, mostly below 75 m and during winter-spring period. Abundance of *D. psilonereia* in Adriatic Sea did not exceed 100 cells L⁻¹. The genus *Dicrakerisma* has been described and reported primarily from the boreal and polar waters, but now it is widespread in all the world oceans. Although *Dicrakerisma* has been encountered in the Mediterranean those are the first findings in the Adriatic Sea. Recent investigations have shown that incoming currents, of either Atlantic/Western Mediterranean or Eastern Mediterranean origin, modify the composition of the plankton community in the South Adriatic and each type of current brings different newcomers.

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OXIDATIVE BURST IN RESPONSE TO PATHOGEN ATTACKS IN A RED ALGA PYROPIA TENERA

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Disease management is the most serious concern for the rapidly growing seaweed aquaculture industry. Oomycete and viral diseases causes serious economic losses in *Pyropia* sea farms every year. Respiratory burst plays an important role in pathogen defence in red algae *Pyropia* species. Histochemical staining and microarray profiles on disease infected *Pyropia tenera* showed that retrograde signaling from cell organelles is involved in the defence mechanism against infection. DAB staining of pathogen-infected *P. tenera* blades showed an accumulation of hydrogen peroxide. Microarray gene profiles showed that respiratory burst oxidase homologues (RBOH) were specifically upregulated in the infected blades of *P. tenera*. As these enzymes are involved in disease resistance, we examined the role of RBOH in defence responses in *P. tenera* using Diphenyleneiodonium (DPI). DPI-pretreated *P. tenera* showed enhanced infection compared to the control, suggesting that the RBOH protein was involved in defence responses to pathogens. These results suggest that *P. tenera* may have signal transduction pathway regulated by RBOH protein in response to pathogens and uses oxidative burst to inhibit the spread of oomycete diseases.

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PHYTOPLANKTON COMMUNITY CHARACTERISTICS OF YALONG BAY IN AUTUMN, 2015

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To understand the status of community structure and diversity of phytoplankton in waters in Yalong Bay of Hainan Province, investigations on phytoplankton at eight stations were conducted in September 2015. Species diversity was

evaluated using Shannon-Wiener diversity index (H'), Pielous species evenness (J') and Berger-Parker dominant degree (Y). In total 74 species (including variety) in 29 genera were identified, in which 43 species in 20 genera belonged to Bacillariophyta, and 30 species in 8 genera for Pyrrophyta, 1 species in 1 genus for Cyanophyta. The dominant species were *Trichodesmium erythraeum*, *Skeletonema tropicum*, *Thalassionema nitzschioides* var. *parva*, *Ceratium horridum* var. *tenuis*, *Chaetoceros affinis* var. *affinis*. The cell abundance of phytoplankton ranged from 0.05×10^4 to 47.67×10^4 cells·L⁻¹, with an average of 15.27×10^4 cells·L⁻¹. H' value ranging from 0.45 to 3.30, J' value ranged from 0.09 to 0.71, Species diversity was very very rich.

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MS-AFLP AND ATP ANALYSIS REVEAL CRYOPRESERVATION-INDUCED STRESS ON GREEN MICROALGAE

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The Culture Collection of Algae at University of Goettingen (SAG) maintains over 2800 strains representing all major groups of microalgae and cyanobacteria. It functions as resource and service infrastructure as well as repository of expert knowledge on identifying, isolating, culturing and ex situ conservation. For indefinite storage, and to circumvent problems associated with serial transfers, cryopreservation at ultralow temperatures is successfully established for 1/3 of SAG strains today. Cryogenic storage is assumed to optimally support genetic and phenotypic stability of organisms. But temperature extremes, cryoprotectants (CPs) and free radical mediated cryoinjury may cause cryostress and genetic alterations. Genetic stability of cryopreserved microalgae has been proven previously using amplified fragment length polymorphism (AFLP). However, it remained unclear if small changes observed

in some cryopreserved strains were due to genetic (mutations) or epigenetic effects (DNA-methylation). Therefore, we analyzed epigenetic integrity in cryopreserved green microalgae using cytosine methylation sensitive AFLP (MS-AFLP using MspI and HpaII enzymes). Several closely related green algae (*Chlorella*, *Micractinium*) as well as model organism *Chlamydomonas* were cryopreserved using optimized protocols. FDA staining revealed different cell survival rates in cryorobust ($\geq 50\%$), intermediate ($\approx 50\%$) and cryosensitive ($\leq 50\%$) strains. Cryopreservation did not significantly change methylation rates between control (precryo) and cryopreserved cultures (postcryo after 2 and 7 wk regrowth). But high variability in methylation patterns revealed epigenetic effects, especially in cryorobust strains. ATP content was measured as a universal physiological stress parameter. ATP levels were higher in cryorobust strains, but generally decreased under cryostress. Cryosensitive organisms were characterized by significantly higher loss of ATP. Full postcryo recovery was only observed in cryorobust organisms, while there was no or low ATP recovery in some intermediate and cryosensitive organisms. Thus, ATP measurements proved suitable for detection of cryopreservation stresses in algae.

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COMMUNITY STRUCTURE AND PRELIMINARY CHARACTERISATION OF THERMOPHILIC HOT SPRING CYANOBACTERIA OF WESTERN SICHUAN, CHINA

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Microorganism inhabiting extreme thermal environments play a crucial role in energy metabolism and matter cycling. Cyanobacteria are a diverse group of microorganisms that are extensively distributed in various ecological niches including hot springs and play an important part in sustaining the productivity of ecosystems through photosynthesis and nitrogen fixation, and accumulating phosphorus as cytoplasmic polyphosphate granules.

Ganzi Prefecture located in western part of Sichuan province of China is transition regions between Tibetan Plateau

and Sichuan Basin. Tectonically, Ganzi is situated at the collision boundary between the India and Eurasia plates. Active new tectonic movement resulted in the abundant hot springs throughout the Ganzi prefecture. The hot springs in Ganzi span a wide range of temperature from 30 to 98°C and were located at high altitude (up to 4200 m above sea level). Therefore, phylogenetically and functionally distinct microbial communities might be shaped by numerous niches in these geothermal areas. However, to date there is no hydrological and microbiological study on hot springs in Ganzi.

The paper will focus on meta 16S analysis of hot spring communities across five thermal sites in Ganzi prefecture. Meta 16S sequences were amplified from microbial mat and hot spring water samples using universal prokaryotic primers specific for V4-V5 region of 16S rRNA gene and sequenced with Illumina MiSeq PE250 platform. Simultaneously culture dependent methods have been used to isolate 132 cyanobacterial isolates capable of growth above 45°. These strains are currently screened using molecular and growth characteristic methods to exclude duplicates.

The primary objective of present study was to census high temperature springs in western Sichuan and to investigate the microbial community composition and diversity in the hot springs using culture-dependent and independent methods. The relationships between biodiversity and physicochemical conditions of the springs were also analysed. This study provided a first insight into the microbial composition in hot springs of western Sichuan, and expanded the current understanding of these niches.

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HOW DEEP IS HOMOPLASY IN ARDISSONEA CRYSTALLINA AND PENNATE DIATOMS?

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Similarity in species of different ancestry resulting from convergent evolution is called homoplasy. Polar centric and pennate diatoms represent fairly different lines of evolution which diverged around 172–219 Ma. Toxariales, comprising among others the genus *Ardissonaea*, belong to polar centrals according to molecular phylogeny. Toxariids developed extremely elongated frustules and a benthic style of life which are characteristic of pennate diatoms. Morphological and ecological similarities are not unusual if one considers homoplasy of Toxariids and the pennate lineage of diatoms. Much deeper convergence has been found in their reproductive features. All centric diatoms whose auxosporulation has been thus far observed had an oogamous mode of sexual reproduction. Syngamy occurs between a uniflagellate sperm and a large immobile ovum. More or less equal-sized gametes are produced by pennate diatoms, but no one case of sperm production has been registered. We were successful in triggering homo- and heterothallic sexual reproduction in *Ardissonaea crystallina* (C. Agardh) Grunow clones maintained in culture. The reproduction turned out to be nonoogamous. Equal-sized gametes, one per gametangium, were formed in the process of gametogenesis. Male gametes produced slender cytoplasmic projections facilitating syngamy, similar to those of some araphid diatoms. However, no flagellated sperms were found. It is interesting that a close relative to Toxariids, *Hydrosera triquetra* Wallich demonstrated oogamous reproduction, usual for centric diatoms. The homoplasy revealed between *A. crystallina* and pennate diatoms is even stronger if one takes into consideration the mechanism of sex determination. Epigenetic sex-determination is typical for centrals; in contrast to this, pennate diatoms have a diplogenotypic sex-determination system. In agreement with this, the breeding system of *A. crystallina* being heterothallic in the base also resembles that of pennate diatoms. Now the challenge is to find molecular differences between *A. crystallina* and its closest oogamous relatives, to explain their divergence in reproduction traits.

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FACTORS CONTROLLING THE DISTRIBUTION OF MARINE BENTHIC DIATOMS ALONG THE COAST OF SOUTH AFRICA

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Diatom (Bacillariophyta) distribution in the oceans is related to the numerous environmental factors, e.g., latitude, type of substrate and physicochemical properties of the water (temperature, salinity, nutrient concentrations, waves, currents, upwelling events, etc.). Although the number of described species reaches over 60,000 taxa, many aspects about their ecological requirements regarding temperature, light or nutrients are still uncertain or unknown. Furthermore, the knowledge of the mechanisms affecting or controlling distribution of the diatom communities and individual species in the ocean is also very vague.

The present study aims to resolve the taxonomic composition and the possible mechanisms controlling the distribution of the marine benthic diatom assemblages along the coast of South Africa and inhabiting (1) a semi-closed Langebaan Lagoon, (2) Saldanha Bay and (3) Tsaarbank – an open Atlantic Ocean coast.

Along with the taxonomic and statistical analysis, we also present results of experiments on temperature effects on diatoms isolated from the Langebaan Lagoon and Tsaarbank. Our taxonomic observations indicate the existence of specific diatom composition in the sheltered and warmer Langebaan Lagoon and Saldanha Bay, which is almost absent in samples from the open ocean coast (Tsaarbank), despite the short distance between sampling stations.

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INTERACTIONS BETWEEN HETEROTROPHIC BACTERIA AND THE OLEAGINOUS, MARINE MICROALGAE NANNOCHLOROPSIS – ESTABLISHMENT OF A TRIPARTITE MODEL- SYSTEM

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A growing number of algal-bacteria interactions have been described. However, very few studies addressed how bacteria interact with commercially important microalgae. In addition, how interactions between different bacteria might influence algal fitness remains largely underexplored. To explore how such microbial interactions affect the algal host, we focused on *Nannochloropsis*, a biotechnologically interesting microalgal genus that is well known for high quality lipid biosynthesis. We investigated whether members of ubiquitous marine bacterial genera, *Alteromonas* and *Marinobacter*, were able to alter the growth of *Nannochloropsis* in laboratory cultures. To test this possibility, we developed a set of co-cultures where bacterial strains were either added separately or in combination. We observed both algicidal as well as growth enhancing effects of co-cultivating single bacterial strains with *Nannochloropsis*. It was interesting that, when both bacteria were present, the growth of the antagonistic bacterium was suppressed resulting in a loss of its algicidal activity. These results show that *Nannochloropsis* can suffer or benefit from the presence of bacteria, either by direct interactions or by the indirect protection against antagonistic bacteria. This tripartite system holds great potential for further exploration of the interactions between oleaginous microalgae and heterotrophic bacteria, potentially leading to valuable applications to enhance the sustainability of commercial mass-cultivation of marine microalgae in industry.

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THE ANNOTATION OF SEA LETTUCE ULVA GENOME: A GREEN ALGAL BLOOMING SPECIES

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Out of 224 published plant genomes up to May 2017, there are only 13% belonging to algae. To broaden our understanding of how plants evolve and not to be limited by land

models, there is a need to sequence species from far neglected lineage. As one of the main multicellular branches in green algae, the sea lettuce *Ulva* is distributed widely in marine and brackish environments around the world. Several unique properties of *Ulva* make it a promising model organism to study: algal morphogenesis, development, interactions with the microbiome, and to provide further evolutionary information for *Chlorophyta*. Here, we report the assembly and annotation of the genome of *Ulva mutabilis*, which is 74 Mbp comprising 676 scaffolds with N50 equal to 267 Kbp. We predicted 10,482 coding sequences (CDSs) using an evidence-based pipeline to reconcile: *ab initio* predictions, predictions from orthologous improvements, coding predictions from enormous RNA-Seq assemblies and evidence from several public resources including UniProt and the *Ulva linza* transcriptome. Benchmarking Universal Single-Copy Orthologs (BUSCO) analysis showed that 86% of eukaryotic BUSCO were classified as complete, and 98% of custom algal BUSCO were identified. Furthermore, we compared the gene models to 1,815 pico-PLAZA core gene families (under *Chlorophyta* division) and revealed that 95% of the core families were well predicted. Annotation of the repetitive sequence resulted in 19% of the genome being masked. The resulting resources serve as the cornerstone of ongoing comparative and functional analyses.

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THE ULVA GENOME: INSIGHTS IN THE BIOLOGY OF BLOOM-FORMING GREEN SEAWEED

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Green algae in coastal environments are dominated by Ulvophyceae of which *Ulva* or Sea lettuce is one of the best-known representatives. The genus is famous for its spectacular algal blooms, which even though not toxic, may smother coastlines and have a negative impact on tourism and the coastal economy. Apart from being a nuisance alga, *Ulva* is also cultivated for human consumption (aonori) and used in integrated multitrophic aquaculture facilities to mitigate nutrient effluents. *Ulva* is also increasingly regarded a complementary model organism to study evolution of

multicellularity in the green lineage. Complete morphogenesis into a blade or tube-like seaweed depends on bacterial interactions, without which the thallus only forms an undifferentiated callus. Transformation protocols enabling functional characterization of genes have been developed. Here we report on the first whole genome sequence of *Ulva*. Thereto, we sequenced and annotated the 74 MB haploid genome of *Ulva mutabilis*.

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IDENTIFYING DRIVERS OF SYMPATRIC SPECIATION IN THE MARINE BENTHIC DIATOM SEMINAVIS ROBUSTA USING METABOLIC ANALYSIS AND WHOLE-GENOME RESEQUENCING

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Diatoms stand out among other groups of microalgae by their extraordinary species diversity. Whereas multiple processes may contribute to reduced gene flow between populations, the key challenge is to understand how reproductive barriers arise between them. We recently discovered

the sympatric occurrence of multiple mating groups of the benthic pennate diatom *Seminavis robusta* in different locations in Belgium and the Netherlands. While sexual reproduction between isolates of the same mating group is highly successful under laboratory conditions, inter-group mating success is significantly reduced. The chemical signaling process involved in mate finding in *S. robusta* has recently been unraveled. Cells of opposite mating types (MT+ and MT-) both produce *sex-inducing pheromones* (SIP+ and SIP-) when their cell size is below a critical sexual size threshold (SST). MT- cells are induced by SIP+ to produce L-dipropine, an attraction pheromone that attracts MT+ cells. Here we test whether divergence of the pheromone signaling is linked to the presence of reproductive barriers in the *S. robusta* species complex. To our surprise, metabolic analyses and comparison of intra- and inter-clade sexual crosses revealed the production of SIP+ and SIP- and the attraction pheromone L-dipropine in all mating groups, suggesting the observed reproductive incompatibility between mating groups must be caused by another factor.

In parallel, whole-genome sequencing was performed to characterize genomic divergence in the *S. robusta* complex using a set of 48 natural isolates (three mating groups, 16 isolates each). The sequences were mapped to a draft *S. robusta* reference genome and initial results reveal considerable genome-wide differences between the three clades, independent of their geographical origin. Genome-wide SNP analysis will allow the identification of highly divergent genomic regions associated with incipient speciation.

Functional annotation of these “speciation signatures” may generate new insights into the mechanism that underlies divergence of this cryptic species complex.

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EUKREF. A COMMUNITY EFFORT TOWARDS PHYLOGENETIC-BASED CURATION OF RIBOSOMAL DATABASES

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In 2001 the first clone library based eukaryotic environmental surveys were published using the 18S rRNA gene as a barcode. Since then a lot of studies have used this approach to describe protistan communities in a wide range of environments. The emergence of high-throughput sequencing (HTS) techniques has made this approach even easier to apply. Consequently, the amount of data retrieved has dramatically increased and our knowledge of diversity

is ever increasing. But there are pitfalls behind the HTS approaches, which require using, and trusting, reference databases to annotate our data. These databases sometimes contain curation errors and other mistakes that potentially alter our overall view of protistan diversity within and across ecosystems. EukRef, the 18S rRNA Collaborative Annotation Initiative is a community-wide effort that addresses these challenges by bringing together people with expertise in diverse eukaryotic lineages to curate 18S rRNA data using phylogenetic methods. Our goal is to assemble a curated reference database spanning the eukaryotic tree of life. This will be a community resource consisting of curated sequences, flexible taxonomy, phylogenetic trees and their underlying sequence alignments. This database will increase the power of HTS-based studies to uncover fundamental patterns in microbial ecology and diversity. Along the way, individual curators are quite likely to identify novel eukaryotic clades and gain new insight into the environmental distribution patterns of eukaryotic microbes.

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ORGANIZATION OF PLASTID GENOMES IN THE FRESHWATER RED ALGAL ORDER BATRACHOSPERMALES (RHODOPHYTA)

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Chloroplast (cp) genomes of seven members of the freshwater red algal order Batrachospermales were sequenced aiming to: describe the characteristics of the cp genomes and compare them with other red algal groups; infer the phylogenetic relationships among these members to try to have a better understanding of the infra-ordinal classification; and raise some possible characteristics that could help to explain the transition from marine to freshwater

habitats. The cp genomes of the Batrachospermales are large, with several cases of gene loss, gene-dense and have highly conserved gene order. The phylogeny based on concatenated nucleotide genome data roughly supports the current taxonomic system for the order. Comparative analyses confirm previous studies of members of the Florideophyceae in that the cp genome in the Batrachospermales is highly conserved, with little variation in gene composition. However, some relevant new features were revealed in our study: genome sizes in members of the Batrachospermales are near the lowest limit reported for the Florideophyceae; the differences in cp genome size within the order are high in comparison to other orders where more than one member was sequenced (Cerariales, Gelidiales, Gracilariales, Hildenbrandiales and Nemaliales); and members of the Batrachospermales have the lowest number of protein coding genes among the Florideophyceae. The interordinal relationships within the Nemaliophycidae are mostly resolved, although the relationship between the freshwater orders Batrachospermales and Thoreaales within the Nemaliophycidae has still to be completely addressed.

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MOLECULAR EVOLUTION AND MORPHOLOGICAL DIVERSIFICATION OF ULVOPHYTES

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In coastal environments green seaweeds (Ulvophyceae) are extremely successful from an evolutionary as well as

an ecological perspective. Ulvophytes display a wide morphological and cytological diversity with unique features among green algae. Morphological types range from unicells and simple filaments to sheet-like and complex corticated thalli. Cytological layouts range from typical small cells containing a single nucleus to giant cells containing millions of nuclei. This cytomorphological differentiation most likely coincided with profound changes in their genomes, and hints to multiple independent evolution of macroscopic thalli growth. The monophyly of this Class have been under discussion; phylogenetic analyses based on limited nuclear datasets or on chloroplast genes failed to converge towards a defined topology. Recently, phylotranscriptomics has been applied to resolve ancient and difficult phylogenetic relationships in various non-model organisms and lineages and to describe the molecular markers associated with the evolution of biological innovations. Here we present a phylotranscriptomic approach to resolve the phylogenetic relationships between Ulvophytes and between the core Chlorophyta lineages and to unveil the molecular features behind their unique cyto-morphological evolution. We produced comprehensive RNAseq data for representatives of each of the major Ulvophytes lineages and we collected sequences of relevant species among the whole green lineage from public repositories. After rigorous benchmarking, we built a robust phylotranscriptomics pipeline that we used to analyze our extensive dataset. First a wide variety of phylogenetic methods were used to build a phylogenetic tree of Ulvophytes and Chlorophyta including all the major lineages. Then, molecular innovations were discovered by clustering the transcriptomes and by inferring orthologous group. The result is a complete new view over the evolution of Ulvophytes.

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PREDATOR CONTROL FOR LARGE SCALE CULTIVATION USING PULSED ELECTRIC FIELD TECHNOLOGY

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Pulsed Electric Field (PEF) processing uses short, high voltage electrical pulses to disrupt cell membranes. The objective was to investigate the application of PEF processing as a low-cost, chemical-free method of microalgae crop protection via mitigation of destructive predator organisms. Fourteen different strains were subjected to a range of field strengths (0–39 mV/cm) and pulse durations (0–20 μ S)

to determine lethal conditions for each strain. Culture viability was determined using four techniques: 1) light and phase contrast microscopy, 2) subjecting PEF-treated cultures to Sytox[®] fluorescent microscopy to evaluate cell membrane permeability, 3) spectrophotometric scans from 300–800 nm to evaluate pigment release resulting from the diminished integrity of cell membranes, and 4) observing subsequent growth after streaking PEF-treated cultures on nutrient-rich agar plates. After this series of unialgal culture viability tests (baseline testing), naturally occurring contaminated cultures were PEF-processed under the range of conditions described above. Our results indicated that PEF treatment successfully killed rotifers, ciliates, amoeba and *Poterochromonas*, a very difficult flagellated algal predator, at field strengths that were non-lethal to *Chlorella vulgaris*. We are the first researchers to demonstrate PEF lethality of *Poterochromonas* without also killing the microalgae culture. In addition, there appears to be a sub-lethal stress effect on some microalgae strains making them more resistant to Sytox[®] staining and potentially enhancing algal growth. Several of the unialgal cultures tested appeared to grow better than the untreated control on nutrient-rich agar plates after being subjected to field strengths of 3–5 mV/cm for 20 μ S. PEF treatment of algal predators appears to be a cost effective, chemical-free approach, which can be applied without damage to the microalgae itself. Future research includes the implementation and operation of Diversified Technologies, including field PEF units on pre-commercial scale open-raceway ponds at AzCATI and open-raceway ponds on-site at commercial partners.

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PATTERNS OF GENETIC DIVERSITY OF THE CRYPTOGENIC RED ALGA POLYSIPHONIA MORROWII (CERAMIALES, RHODOPHYTA) SUGGEST MULTIPLE ORIGINS OF THE ATLANTIC POPULATIONS

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The red alga *Polysiphonia morrowii*, native to the North Pacific (Northeast Asia), has recently been reported worldwide. To determine the origin of the French and Argentine populations of this introduced species, we compared samples from these two areas with samples collected in Korea and at Hakodate, Japan, the type locality of the species. Combined analyses of chloroplastic (*rbcl*) and mitochondrial (*cox1*) DNA revealed that the French and Argentine populations are closely related and differ substantially from the Korean and Japanese populations. The genetic structure of *P. morrowii* populations from the South Atlantic and North Atlantic, which showed high haplotype diversity compared with populations from the North Pacific, suggested the occurrence of multiple introduction events from areas outside of the so-called native regions. Although similar, the French and Argentine populations are not genetically identical. Thus, the genetic structure of these two introduced areas may have been modified by cryptic and recurrent introduction events directly from Asia or from other introduced areas that act as introduction relays. In addition, the large number of private cytoplasmic types identified in the two introduced regions strongly suggests that local populations of *P. morrowii* existed before the recent detection of these invasions. Our results suggest that the most likely scenario is that the source population(s) of the French and Argentine populations was not located only in the North Pacific and/or that *P. morrowii* is a cryptogenic species.

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REVISITING AUSTRALIAN FRESHWATER ECTOCARPUS FROM THE HOPKINS RIVER: DISTRIBUTION, ABIOTIC ENVIRONMENT AND ASSOCIATED CULTIVABLE MICROBIOME

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Ectocarpus is a genus of common marine brown algae. In March 1995 a strain of *Ectocarpus* was discovered growing in a freshwater environment just above the Hopkins River Falls, Victoria, Australia, approximately 15 km inland (elevation 26 m). The corresponding isolate (CCAP

1310/196) is one of very few available freshwater brown algae and the only one belonging to the genus *Ectocarpus*. It has since been used as a model to study acclimation and adaptation to low salinities. However, so far virtually nothing is known about the natural distribution of *Ectocarpus* in the area around Hopkins River Falls, nor do we know whether there is a stable population of *Ectocarpus*. Furthermore, the associated microbiome, which has been shown to play a role during the acclimation to low salinities, as well as the algal strain itself, may have been strongly impacted by the long period of cultivation in the laboratory.

Twenty-two years after this original finding we have searched for *Ectocarpus* along the entire Hopkins river as well as in neighboring rivers and along the coastline. We found abundant occurrences of *Ectocarpus* at three sites above Hopkins River Falls at 20 to 60 km distance from the sea. The osmolarity of the water at these sites ranged from 70–170 mOsmol, and further chemical analyses of water samples are ongoing. Two additional *Ectocarpus* isolates were obtained from nearby marine sites via germling emergence experiments. No *Ectocarpus* was found at the original site, which was now dominated by diatoms, nor in any of the other sampled rivers. As for bacteria, over 100 strains were isolated in situ from ground *Ectocarpus* filaments. Their molecular identification, as well as metabarcoding of the bacterial community associated with *Ectocarpus* thalli in the field, is still ongoing.

Our results confirm that *Ectocarpus* has stably colonized Hopkins River, an inland, low salinity habitat. The freshly isolated algal strains, as well as the isolated bacteria, offer new possibilities to study adaptations of *Ectocarpus* to low salinities, and in particular the impact of the microbiome on its capacity to grow in this environment.

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PHYLOGENOMICS RESOLVES THE EARLY RAPID RADIATION OF THE RHODOMELACEAE (RHODOPHYTA) AND INFORMS TRIBAL-LEVEL RECLASSIFICATION

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The introduction of molecular tools has revolutionized the study of red algal systematics throughout all taxonomic levels over the last 25 years. Most of the research employed a single or a few molecular markers, with the inherent limitation that it does not provide enough data to resolve many phylogenetic relationships. The recent development of high-throughput sequencing techniques allows us to sequence easily the complete chloroplast genome. The application of genome sequences in resolving challenging phylogenies has been demonstrated clearly in land plants, but red algae are still underrepresented in organellar genomes databases. In this work, we attempted to resolve the phylogenetic relationships among major lineages of the most diverse red algal family, the Rhodomelaceae, using a phylogenomic approach. We assembled a dataset consisting of the 194 genes of the chloroplast genome for 45 (42 newly determined) Rhodomelacean species and 11 (10 newly determined) Ceramiales (outgroup). The phylogenetic analysis produced well resolved trees, with full bootstrap values for the majority of nodes. Subsequently, we assembled a second dataset including *rbcL*, *cox1* and 18S sequences for 407 species, and constructed a phylogeny constrained using the genome-scale tree as a backbone. Both phylogenies along with morphological characters relevant to the delineation of tribes were interpreted in order to re-evaluate the tribal classification of the Rhodomelaceae. In addition to the 14 tribes previously recognized, we proposed five new and one resurrected tribe. This work demonstrates the strength of chloroplast genome data to resolve challenging phylogenies in the red algae and its application to resolve classification issues.

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MOSAIC ORIGINS AND COMPLEX FATES OF OCHROPHYTE PLASTIDS REVEALED THROUGH AN ANCIENT ORGANELLE

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Plastids are supported by a wide range of proteins encoded within the nucleus, that are imported from the cytoplasm. These plastid-targeted proteins may originate from the endosymbiont, the host, or other sources entirely. Here, we identify and characterise 770 plastid-targeted proteins that are conserved across the ochrophytes, a major group of algae including diatoms, pelagophytes and kelps, that possess plastids derived from red algae. We show that the ancestral ochrophyte plastid proteome was an evolutionary chimera, with 25% of its phylogenetically tractable proteins deriving from green algae. We additionally show that functional mixing of host and plastid proteomes, such as through dual targeting, is an ancestral feature of plastid evolution. Finally, we detect a clear phylogenetic signal from one ochrophyte subgroup, the lineage containing pelagophytes and dictyochophytes, in plastid-targeted proteins from another major algal lineage, the haptophytes. This may represent a possible serial endosymbiosis event deep in eukaryotic evolutionary history.

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NEEDLES IN WATERY HAYSTACKS: FINDING NOVEL MOLECULES INDUCING DIATOM BIOFILM FORMATION, EXCRETED BY BACTERIA

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Achmanthidium minutissimum (Kützing) Czarnecki is a pennate diatom found globally in freshwater environments where it persists in photoautotrophic biofilm communities, complex habitats comprising of a diverse array of microalgae and bacteria, which poses challenging questions regarding interspecies communication. Meanwhile, the formation and subsequent removal of such biofilms on submerged man-made surfaces incurs significant costs globally. The axenification of *A. minutissimum* cultures revealed that extracellular polymeric substance (EPS) secretion, leading to capsule and biofilm formation, depended on the presence of bacteria. Therefore, strains of bacteria were isolated from photoautotrophic biofilms, at the same location from which *A. minutissimum* was isolated. Subsequent co-culture experiments identified a novel strain of bacteria (S32), phylum Bacteroidetes, as the most effective

isolate at inducing EPS secretion. EPS secretion could be quantified using a crystal violet calorimetric method, and an automated, medium throughput method was developed with the Konstanz University Screening Centre, which allowed precise quantification of biofilm density. Using this method, the authors demonstrated that an excreted signal from S32 was responsible for the induction of diatom EPS secretion. Further analyses showed that this signal was probably a small molecule rather than a polypeptide, based on the bioactivity of solid phase extracts and heat treated samples. Further purification and analysis is ongoing, and indicates a relatively non-polar metabolite is responsible for biofilm induction. In order to investigate the genetics of the of the bacterium S32, whole genome sequencing was conducted, including gene-calling and annotation using the JGI Integrated Microbial Genome/Expert Review browser. A survey of the bacterium's biosynthetic machinery indicated that previously known metabolites affecting diatom behaviour and biofilm formation, including cobalamin (vitamin B12), indole-3-acetic acid (auxin) and N-acyl-homoserine lactones, are not synthesised by S32. These results demonstrate that further studies are required to understand the mechanisms and key environmental signals underlying biofilm formation.

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SELECTION AND DOMESTICATION INFLUENCES ON THE WILD AND CULTI- VATED SACCHARINA JAPONICA (LAMINARIALES, PHAEOPHYCEAE) GENOMES

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Saccharina japonica is the most successful domesticated brown seaweed in marine aquaculture in the world. The annual output of this species in China corresponds

to 98.3% of world kelp production. Using whole-genome shotgun sequencing technology, we conducted *de novo* assembly of one wild *S. japonica* accession, and resequenced 32 wild and cultivated accessions spanning the cold, cool and warm regions along the coast of North Pacific Ocean. It is revealed that there has been strong selection on frond length (FL) and frond thickness (FT) in cultivated *S. japonica*. Moreover, several genes in response to heat-tolerance are found within different domesticated regions from the north and south in China, respectively. It is concluded that environmental factors, such as temperature, have played an important role in the domestication process. Our data provide valuable information for further analysis of wild *S. japonica*, and will facilitate kelp selection, breeding and quantitative trait analysis.

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VARIABILITY IN STABLE ISOTOPE SIGNATURES OF SOUTH AFRICAN LAMINARIALES: IMPLICATIONS FOR KELP FOREST FOOD WEB STUDIES

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Stable Isotope Analysis (SIA) has become an indispensable tool for investigating food web characteristics, with particular focus on trophic structure and functioning. Variability of basal food web components is poorly understood and often neglected, despite being a key assumption of food web models. Kelp forests provide an excellent ecosystem to evaluate the scale and implications of this variability as they exhibit “bottom-up” control. In South Africa, kelp forests are primarily formed by two species, *Ecklonia maxima* and *Laminaria pallida*, which occur along the temperate regions of the coastline. This study highlights the natural variability of stable isotope signatures ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$), at different scales, within these two species. Kelp tissue was collected from different parts within a single plant (hold-fast, stipe, primary blade and frond) and at different positions along the length of a single frond (from primary blade to frond tip). Representative samples were also collected at nine different geographical localities between Port Nolloth

and Betty’s Bay. A range of 1.65‰ and 3.75‰ was found within an *E. maxima* plant, for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ respectively. The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ range within a *L. pallida* plant was 1.52‰ and 4.21‰ respectively. Consistent variability patterns along the length of a single frond in both species, for both isotopes were revealed. Across localities, *E. maxima* and *L. pallida* were highly variable in $\delta^{13}\text{C}$ (9.37‰ and 11.22‰ respectively) and $\delta^{15}\text{N}$ (3.44‰ and 4.51‰ respectively). Site and season were major contributors to overall variability for *L. pallida*, whereas season and within-site variability explained most variance within *E. maxima*. Although the cause of the variability is not entirely understood, it can create erroneous conclusions if not considered when modeling kelp forest food webs. Therefore, the nature and scale of variability within kelps is imperative for gaining an accurate understanding of South African kelp forest food web functioning.

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DO DIATOM-DERIVED POLYUNSATURATED ALDEHYDES IMPACT DIATOM-ASSOCIATED MICROBIOMES?

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One of the as yet unresolved questions in microalgae chemical ecology is the role(s) of diatom-derived polyunsaturated aldehydes (PUA), whose biosynthesis increases with culture age and in response to nitrogen stress. PUA are semiochemicals that, through selectional pleiotropy, are mooted to combat metazoan grazing, deter competition from other microalgae, be bactericidal, and even trigger the onset of diatom sexual reproduction. Despite hypotheses that PUA are antimicrobial, the published data are equivocal. A recurrent limitation of PUA studies is the use of dosages in excess of known environmental levels, arguably weakening any ecological interpretation. As such, the current study investigated the microbiome associated with cultures of two contrasting *Skeletonema marinoi* strains – a known PUA-producer versus a PUA-free strain – when cultured under three nitrate levels over the course of a full batch culture cycle. Moreover, the response of each culture microbiome was also studied when challenged with PUA at ecologically relevant concentrations (86 nM octadienal

and 290 nM heptadienal). Using next generation sequencing, 741 OTUs were identified, from 27 phyla, 38 classes, 50 orders, 102 families and 147 genera, excluding unclassified results. The study revealed complex interplays within the diatom culture microbiomes, influenced by culture age, nitrogen state and, importantly, the availability of PUA, thus providing evidence that PUA are important in structuring the bacterial communities associated with bloom forming diatoms.

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BREAKING BAD: OPPORTUNISTIC BACTERIAL PATHOGENS OF SEAWEEDS

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Macroalgae form a diverse and ubiquitous group of photosynthetic organisms that play an essential role in many aquatic ecosystems, yet till recently very little was understood with respect to their associated microbiota. While symbiotic interactions with marine microbes are essential for macroalgae, we now also know that microbes can be responsible for negative outcomes, such as disease of the host. A major challenge, however, is to link specific microbial pathogens to particular disease events or phenotypes. Moreover where pathogens have been identified little is known about the specific mechanisms of host interaction. To overcome these challenges, we have used a combination of microbial ecology, (meta-) genomics and classical microbiology to unravel the causes and mechanisms of bleaching disease in the model the red macroalga *D. pulchra*. We have found multiple and opportunistic pathogens are capable of causing bleaching of *D. pulchra*, including the previously characterised Roseobacter clade members (*Nautella* sp. R11 and *Phaeobacter* sp. LSS9), two *Aquimarina* spp., an *Alteromonas* sp. and an *Agarivorans* sp. Profiling of the bacterial community *D. pulchra* post infection with these pathogens further suggests a role for microbial dysbioses in this macroalgal disease. Genome and mutational analysis of specific bacterial pathogens has identified bacterial traits, including

those related to quorum sensing, colonisation, oxidative stress resistance and carbohydrate metabolism as potentially common drivers of host-pathogen interactions. These studies contribute to our understanding of the microbial mechanisms that mitigate health and disease in macroalgae and the potential processes by which otherwise harmless seaweed symbionts can opportunistically “break bad” and cause disease.

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METABARCODING OF HAPTOPHYTES

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Haptophytes are one of the major groups of primary producers in the ocean. Some haptophytes, such as members of the genera *Emiliana*, *Gephyrocapsa*, *Phaeocystis* and *Prymnesium* form extensive blooms that can affect the global carbon balance, or cause fish kills with ecological and economic impact. Calcifying haptophytes, the coccolithophorids, have species-specific coccoliths that can be distinguished in the scanning electron microscope. However, a large fraction of haptophyte species are tiny and covered by thin, organic scales, and require transmission electron microscopy for identification, which is less quantitative. Knowledge of haptophyte diversity and distribution therefore remains fragmentary. High-throughput sequencing platforms have facilitated fast and cheap deep-sequencing, enabling recovery of rare species, and have made it possible to investigate spatial and temporal dynamics in greater detail. In this talk I ask how the metabarcoding approach has changed our views of haptophyte diversity and dynamics. What is the extent of the unknown diversity revealed by metabarcoding? Which groups are most diverse? What new information about the spatial and temporal dynamics of haptophytes has been obtained?

To interpret metabarcoding results, a comprehensive reference database is essential. Therefore I first look at the extent of diversity revealed by environmental clone libraries and Sanger sequencing. A recent 18S rDNA phylogeny including all cultured species and environmental sequences longer than 800 basepairs reveals several novel clades that consist only of environmental sequences, and that are divergent enough from established clades to represent new classes. When classified against such a reference database, metabarcoding OTUs can tell us more about the distribution of

cultured and sequenced species, and also about the novel clades. To show this, I will use examples both from temporal studies at one site (Oslofjorden) and studies of spatial distribution (European coastal waters and Arctic Ocean north-west of Svalbard).

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PHOTOSYSTEM I IN CHROMERID ALGAE: LOSSES, GAINS, AND DAMAGE CONTROL

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The light-dependent reactions of oxygenic photosynthesis are carried out by multiprotein complexes embedded in the thylakoid membranes of cyanobacteria and plastids, namely photosystems I and II (PSI and PSII), cytochrome b6f, and ATP synthase. While this machinery is conserved throughout photosynthetic organisms, the component subunits of the PSI, PSII, cytochrome b6f and ATP synthase complexes vary between lineages. However, these differences have been explored in relatively few taxa. We carried out complementary biochemical and in silico studies on the thylakoid membrane complexes (concentrating on PSI) of *Chromera velia* and *Vitrella brassicaformis*, unicellular algae that represent the closest photosynthetic relatives of the apicomplexan parasites and possess complex red plastids (i.e. those derived from a secondary or tertiary endosymbiotic event involving a red algal prey cell). While *C. velia* and *V. brassicaformis* lack the multiple PSI subunits present in most other taxa with complex red plastids, they have gained: 1) additional subunits that do not appear to have homologs in sequence databases, and 2) one (*Vitrella*) or two (*Chromera*) iron superoxide dismutases (FeSODs) that appear to be more or less permanently associated with PSI, suggesting an important role for the water-water cycle in both taxa.

Moreover, light harvesting fucoxanthin-chlorophyll binding proteins (FCPs) with strong associations to PSI could be identified. Phylogenetic analysis of FeSOD sequences suggests (albeit without statistical support) that the PSI-FeSODs in *C. velia* and *V. brassicaformis* are closely related to one another but are not monophyletic, branching

among sequences from other myzozoans. Phylogenetic analysis of FCPs indicates that only one PSI-associated FCP in *C. velia* is closely related to a PSI-FCP from *V. brassicaformis*; the remaining *Chromera* PSI-FCPs are related to PSI-FCPs from other taxa bearing red plastids, while the remaining *Vitrella* PSI-FCPs branch with sequences that have not been identified as strongly associated with PSI.

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FEED STIMULANTS FROM GREEN ALGA ULVA FOR SEA URCHIN TRIPNEUSTES GRATILLA

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Secondary metabolites play a fundamental role in mediating ecological interactions for marine plants. Much evidence of this chemical mediation comes from plant/herbivore interactions, where it is clear that some metabolites act as allelochemicals. However, other ecological interactions such as feed stimulants or attractants are less well known. In South Africa, aquacultured sea lettuce (*U. armoricana*) is known to elicit phagostimulatory responses in the sea urchin *T. gratilla*, with several studies showing a feeding preference for this alga. The present study investigated the feeding preference of *T. gratilla* for several *Ulva* fractions with the aim of identifying chemical compounds that may act as a feed stimulant or attractant. The touch and feed preferences of *T. gratilla* were investigated for nine *Ulva* fractions (F1, F2, F3, F4, F5, F6, F7, F8 and F9; obtained from step gradient SI-gel column), in a circular tank for 75 minutes using a modified version of the 'Avicel' plating technique. We found that F9 and F8 were mostly preferred in both feeding preference tests followed by F6, F5 and F4. Digalactosyl diacylglycerol (DGDG) and monogalactosyl diacylglycerol (MGDG) were isolated from F9 and F8 respectively while the presence of methyl palmitate was observed in F5 and F4. Results show clear evidence of the presence of compounds in this alga that may act as a feed stimulant or attractant for the sea urchin *T. gratilla*.

ENVIRONMENTAL CONTROLS ON THE ELEMENTAL COMPOSITION OF THE COCCOLITHOPHORE *EMILIANIA HUXLEYI*

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A series of semi-continuous incubation experiments were conducted with the coccolithophore *Emiliania huxleyi* strain NIWA1108 (Southern Ocean isolate) to examine the effects of five environmental drivers (nitrate concentration, phosphate concentration, irradiance, temperature and pCO₂) on the physiology of *E. huxleyi*. A series of dose response curves for the cellular elemental composition of *E. huxleyi* were fitted for each of the five drivers across an environmentally representative gradient. The importance of each driver to setting the elemental composition of *E. huxleyi* was ranked using a semi-quantitative approach. The percentage variation in elemental composition arising from the change in each driver between present day and model-projected conditions for the year 2100 were calculated. Temperature was the most important driver controlling both cellular particulate organic carbon and inorganic carbon content, whereas nutrient concentrations were the most important regulator of cellular particulate nitrogen and phosphorus of *E. huxleyi*. In contrast, elevated pCO₂ had the greatest influence (decreasing) on cellular particulate inorganic carbon to organic carbon ratio. Our results indicate that the different environmental drivers each play specific roles in regulating the cellular elemental composition of *E. huxleyi* with wide-reaching implications for coccolithophore biogeochemistry.

TEMPERATURE, GROWTH METRICS, AND THE UNIMODAL SIZE SCALING OF PHYTOPLANKTON GROWTH

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Previous studies have shown that phytoplankton growth rates peak at intermediate cell sizes, contrary to predictions made by the metabolic theory of ecology. It has been suggested that the unimodal relationship between phytoplankton cell size and growth rate may arise because the smallest species are adapted to warm temperatures. A related issue is the possibility of this size scaling pattern is being sensitive to the specific metric used to determine growth rate. To test whether temperature affects the unimodal size scaling pattern, we used a double approach: 1) growing batch cultures of several species at two different temperatures (18°C and 25°C) to measure their growth rate, and 2) using thermal response models available in the literature to estimate the growth of each species at different temperatures. To assess the sensitivity of growth rate estimates to the metric used, we calculated growth rates based on experimental data of abundance, fluorescence, chlorophyll, carbon and nitrogen content. Our results show that phytoplankton growth rates peak at intermediate sizes at both 18°C and 25°C. The unimodal pattern prevails irrespective of the growth metric employed. However, growth rate estimates based on fluorescence and chlorophyll tend to exceed those based on carbon and nitrogen, which reflects consistent patterns in the variability of cellular composition.

AN EVOLUTIONARY IMPRINT ON THE MACROMOLECULAR COMPOSITION OF MICROALGAE

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The elemental stoichiometry of microalgae reflects their underlying macromolecular composition and influences

competitive interactions among species and the biogeochemical cycling of elements. A hierarchical Bayesian analysis of data compiled from the literature provides a new estimate of the macromolecular composition of microalgae. Under nutrient-sufficient, exponentially growing conditions, median microalgae macromolecular composition is 32.2% protein, 17.3% lipid, 15% carbohydrate, 17.3% ash, 5.7% RNA, 1.1% chlorophyll-a, and 1.0% DNA, as percent dry weight. There are significant phylogenetic differences in macromolecular composition undetected by previous studies due to small sample sizes and high inherent variability in the macromolecular pools across species. The phylogenetic differences in macromolecular and elemental composition appear to reflect adaptations in cellular architecture and biochemistry; specifically, in the cell wall, the light harvesting apparatus, and storage pools.

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MASS EUKARYOTIC MICROBIOMES IN CHANGING MARINE BIOREGIONS REVEAL NOVEL OOMYCETE DIVERSITY

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Macro-algal samples collected from the Falkland Islands, Baffin Island and Ascension Island (2009–2012) have recently been the subject of a molecular survey to screen for little reported associated eukaryotic organisms, specifically potential oomycete pathogens. A portion of the SSU rRNA was amplified with both eukaryote-specific and oomycete tailored primers, barcoded, multiplexed and sequenced with 454 technology. Data mining of this has begun, with the identification of Operative Taxonomic Units (OTUs) at 97%. Over 60 oomycete specific OTUs have been identified, split in to three major groups. The first group (Oom1) has several OTUs with a high blast affinity (>97%) to *Eurychasma* and is therefore thought to resemble the diversity of the genus, as yet unrecorded in any other study.

The other two groups (Oom2+3) appear to be generally unrelated to other oomycetes sequenced at this locus. Oom2 appears to be rooted by an OTU with high affinity to the diatom pathogen, *Lagenisma* and is exclusively detected from samples originating in Baffin Island. Oom3 has no high affinity to any oomycete sequence and therefore appears to be a novel clade, of unknown affinity. It would be of interest to attempt to identify these organisms in the field, with a technique such as fluorescence in situ hybridization, to determine which organism may play host to these novel OTUs.

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STRUCTURE OF EPIPHITYC BACTERIAL COMMUNITIES (EBC) ASSOCIATED WITH MACROCYSTIS PYRIFERA IN SITES WITH DIFFERENT NITROGEN AVAILABILITY

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Macrocyctis pyrifera is an ecologically important macroalgae with a wide distribution in the coasts of Chile encompassing a wide gradient of environmental factors. Although there are studies on the macrofauna associated with these macroalgae, the interaction with their CBE, under different environmental conditions, has been little explored. This study analyzed the structure of CBE of *M. pyrifera* in sites with presence and absence of sources of nitrogen enrichment (N) in northern and southern Chile during spring and summer. Bacterial diversity was obtained using the V3 and V4 regions of the 16S rRNA amplicon by the Illumina MiSeq sequencing platform. The diversity α showed differences between the CBE's of the macroalgae and the surrounding seawater. The specific richness and relative abundance of Operational Taxonomic Units was higher in seawater than in CBE's, while bacterial uniformity was high in both substrates. 21 bacterial phyla were identified, being *Proteobacteria* and *Bacteroidetes* that comprised 90% of total diversity. The most abundant bacteria at the genus level were *Citromicrobium* (79.8%) and *Novosphingobium* (9.4%), in CBE's, and *Flavobacterium* (10%)

and *Candidatus pelagibacter* (8.2%) in water sea. In addition, during the summer, the abundance of *Citromicrobium* in CBE's was higher in sites with low concentration of N, whereas, in sites with high concentration, it remained like the spring season. This analysis suggests that there are bacterial phyla commonly associated with macroalgae and a genus could be strongly associated with *M. pyrifera* exposed to variations in the availability of N.

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TERRESTRIAL MICROALGAE ON ROCK SURFACES AS DRIVERS OF BIOGENIC WEATHERING

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The role of algae/cyanobacterial components of microbial communities associated with openly exposed granite rock surfaces are being studied within a German/Chilean research program, "EarthShape", across four defined study areas in the Coastal Cordillera in Chile representing a gradient in aridity. Illumina MiSeq paired ends sequencing targeting the SSU and ITS2 rRNA genes was performed. Complemented by numerous cultured isolates many green algae species and cyanobacteria morphoforms have congruently been recovered by both approaches. Many algal/cyanobacterial OTUs were left unidentified indicative of putative new species not recovered so far. Green algae known as lichen photobionts exhibited the largest microalgal diversity whereas almost no free-living microalgae were recovered. This was in concordance of lichens being the most dominant life form in the studied arid habitats. Clear distributional patterns were revealed along the gradient of arid

climate. The least arid climatic zone exhibited the largest green algal diversity, while the most arid zones appeared to be hotspots of prokaryote diversity. The cyanobacteria exhibited the highest diversity in the arid zone in the North, being confined to unicellular forms. Cyanobacteria were almost absent in the Mediterranean zone. Cyanobacteria diversity increased in the least arid zone in the South, but was then confined to filamentous forms. The large diversity of symbiotic algal/cyanobacteria may have resulted from its success in fuelling heterotroph fungi and bacteria with the energy for the decay and remineralization of rock. It follows that microalgae may have an important but indirect role in the formation of soil from rock and thus shaping the Earth surface.

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THE GENUS *PLACOMA* (ENTOPHYSALIDACEAE, CHROOCOCCALES, CYANOPHYTA/CYANOBACTERIA) FROM JAPAN-TAXONOMY AND MORPHOLOGY

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The genus *Placoma* is a benthic blue-green taxa, attaching on rocks in the rocky shore. It was established by Bornet & Thuret (1876) for having a lump-shaped thallus composed of a lot of cells. Currently 8 taxa are taxonomically effective, and among them 2 taxa are reported in Japan. However, this genus has never been examined at the morphological point of view. The present study aims to a detailed morphological observation of the genus *Placoma* from Japan. In addition, we make the taxonomic comparison with the genus *Hydrococcus* closely related to the genus *Placoma*.

Placoma micrococcum (Hansgirg) Umezaki et M. Watanabe: thallus saccular, yellow-brown, 0.2–0.7 cm in diameter. Inner Cortical cells 0.7–4.2 μm wide, 1.3–6.4 μm long, arranged radially. Mucilagenous yellow-brown. Inner cells 0.2–3.6 μm wide, 1.0–4.0 μm long, arranged irregularly. Growing on rocks in supralittoral to upper littoral zones.

Placoma sp. [described as *Placoma adriaticum* (Hauck) Umezaki et M. Watanabe]: thallus lump-like, olive-green, 0.1–0.3 cm in diameter. Cortical cells 1.0–9.5 μm wide, 1.1–7.9 μm long, arranged radially. Mucilagenous, colorless. Inner cells 0.6–5.9 μm wide, 0.8–6.3 μm long, arranged

irregularly. Epiphytic on a brown alga *Myelophycus simplex* at the middle littoral zone. *Placoma* sp. is significantly different from *P. adriaticum* by the sizes of cortical and inner cells and the attached substrate (*P. adriaticum*: on a red alga *Pterocladia capillacea* from the Adriatic Sea)

Hydrococcus cf. *rivularis* Kützing: thallus lump-like, olive-green, 0.1–0.3 cm in diameter. Cells do not differentiate between cortical and inner layers, 0.7–5.4 µm wide, 1.3–7.9 µm long. Epiphytic on *Corallina pilulifera* at the lower littoral zone in Japan. This taxa is very similar to the species of the genus *Placoma* in the gross morphology, but it make no differentiation of the cells.

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LIFE CYCLES IN PHOTOSYNTHETIC RELATIVES OF APICOMPLEXANS

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Environmental sequencing revealed that core apicomplexans are nested within lineages with a diversity of trophic strategies, including parasitic, predatory, and phototrophic. We investigated life cycles of algal relatives of apicomplexans, *Chromera velia* and *Vitrella brassicaformis*. We have identified isogametic sexual behavior in *V. brassicaformis*, exhibiting a fusion of two motile cells. In contrast, *C. velia* does not seem to utilize cell fusion in any stage of its life cycle. Strikingly, zoospores of *C. velia* possess an apical complex-like structure composed of tubulin, possibly to invade corals; this “pseudoconoid” was not found in zoospores of *V. brassicaformis*. Genetic repertoires of these two species reflect these differences. *Chromera* lacks core meiotic genes and *Vitrella* lacks some genes for apical complex components, such as RING2 and Myosin H. Based on life cycle complexity in apicomplexans and their eukaryotic sister groups, we suppose that the common ancestor of *Vitrella*, *Chromera*, and the apicomplexans had a complex life cycle and cell biology. Some of the extant species, however, underwent a reduction of their cellular complexity possibly to better suit their ecological needs. These results may indicate that a complex ancestral battery of biological processes

could have enabled apicomplexans to establish dioxenous parasite-host interactions.

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STUDY OF THE EFFECT OF CADMIUM ON THE GROWTH AND BIOCHEMICAL COMPOSITION OF DUNALIELLA SALINA

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Microalgae are a source of many compounds that can be used in industry. Synthesis of such compounds in microalgal cells can be amplified under stress conditions. Exposing the microalga *Dunaliella salina* to cadmium metal can induce cell stress and the synthesis of target products in culture. In this study, the potential for *D. salina* to produce diverse biocompounds (pigments, lipids, proteins, glucide) on exposure to cadmium (0.3, 0.7, 2 and 10 ppm) is evaluated. The biochemical composition of *D. salina* was determined as a function of the concentration cadmium and the growth stage. *D. salina* exhibited a fast growth rate of 1×10^7 cells ml⁻¹ in absence of cadmium. At 10 ppm Cd, and in the stationary growth phase, the algal biomass consisted of approximately 111 mg l⁻¹ protein, 261 mg l⁻¹ carbohydrate and 19% lipid. The results also showed that the contents of these constituents increased in the presence of cadmium and depended closely on the culture conditions. Thus, it is possible to direct the cellular metabolism of *D. salina* towards the production of the desired metabolite(s) by selecting the appropriate environmental conditions and the time of the harvest of the algal biomass. *D. salina* can also be used for bioremediation purposes because of its large capacity to accumulate cadmium.

THE BEST IS YET TO COME: MOLECULAR SYSTEMATICS OF GIBSMITHIA (DUMONTIACEAE, RHODOPHYTA)

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The genus *Gibsmithia* was erected to accommodate a species with the peculiar combination of gelatinous lobes rising from cartilaginous stalks. Based on *G. hawaiiensis* from Hawaii, the genus remained monotypic for over 20 years, when additional species were described from Australia. While *G. hawaiiensis* is unique for its furry appearance, its non-hairy congeners can be clearly discriminated by the branching pattern of the gelatinous thallus: rosette clusters of short flattened branches in *G. dotyi*, subdichotomous long cylindrical branches in *G. womersleyi*, and very irregularly branched lobes in *G. larkumii*. Species of *Gibsmithia* have been reported from the Indian Ocean and central and western Pacific, with *G. hawaiiensis* presenting the widest distribution, and *G. womersleyi* occurring only in South and Western Australia. Genetic studies reveal that *Gibsmithia* is more diverse than previously reported. The genus is monophyletic with two main groups: one comprising the hairy species, i.e. the *G. hawaiiensis* complex, and another comprising non-hairy species, including *G. dotyi* and *G. larkumii*. The analyses show that the *G. hawaiiensis* complex encompasses nine distinct species sharing a similar habit. Four of those species can be distinguished based on morphological characters: *G. hawaiiensis* sensu stricto, the recently described *G. eilatensis* and other two new species. *G. dotyi* and *G. larkumii* also seem to represent a complex of cryptic or pseudo-cryptic species, comprising about 10 different lineages. Some observed specimens are non-fertile, while others are only available as dried herbarium specimens or preserved in silica-gel, and their reproductive

characters can not be ascertained with confidence. New records of the genus are provided for Tanzania, Madagascar, Europa Island, Seychelles, the Red Sea (Israel and Egypt), Malaysia, Indonesia, Philippines, Japan, Guam, and French Polynesia. Additional studies are underway to describe the new species and to assess the large-scale phylogenetic and biogeographic patterns in *Gibsmithia*.

HIDDEN DIVERSITY IN THE OOMYCETE GENUS OLPIDIOPSIS IS A GLOBAL THREAT TO RED ALGAL CULTIVATION

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The oomycete genus *Olpidiopsis* encompasses pathogens of red seaweeds, including the most economically damaging disease in *Pyropia* (ex-*Porphyra*) farms in Asia. Here we identified three new *Olpidiopsis* species: *Olpidiopsis palmariae*, *O. muelleri*, and *O. polysiphoniae* spp. nov. as well as a Scottish variety of *O. porphyrae*, a devastating pathogen only reported in Japanese seaweed farms, which is also described as *O. porphyrae* var. *scotiae*. Importantly, two of the new species infected *Porphyra* and *Palmaria* sp., which are the subjects of extensive farming trials in Europe and North America. To further assess the extent of undescribed *Olpidiopsis* diversity and the threat it might pose to aquaculture in different regions, we screened targeted metagenomes of *Porphyra umbilicalis* blades collected on the West and East Northern Atlantic shores (JGI proposal ID 946). Four different *Olpidiopsis* OTUs were detected on at least 5 out of 9 *Porphyra* blade metagenomes, illustrating the prevalence and diversity of these parasites in wild *Porphyra* populations. Finally, we extended our screening efforts to global metabarcoding campaigns, revealing over 700 new

sequences attributable to *Olpidiopsis* with a worldwide distribution. Close relatives of the Korean *O. pyropiae* are reported for the first time in Europe and the United States. In the light of our restricted sampling, our results highlight the diversity and abundance of *Olpidiopsis* worldwide. In the context of worsening impact of *Olpidiopsis* pathogens in Asia, this worldwide distribution should be treated as a serious threat to the global seaweed industry and wild red algal populations. Our data call for more efforts towards the documentation of these pathogens, and for adequate biosecurity measures to be developed.

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DEFENCE REACTIONS OF BROWN ALGAE AGAINST THE OOMYCETE PATHOGENS EURYCHASMA AND ANISOLPIDIUM

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The fundamental role of microbes in the physiology, development, ecology and evolution of algae is now well established, yet the detailed mechanisms of these interactions often remain to be elucidated. The oomycetes *Eurychasma dicksonii* and *Anisolpidium ectocarpii* are two obligate intracellular pathogens with a broad host range that we are using to investigate the immune responses of brown algae. We find that resistance to infection by *Eu. dicksonii* is mediated by the hypersensitive death of the algal cells attacked.

This response is accompanied by the deposition of beta-1,3-glucan in the cell wall and of blue-fluorescent metabolites, the production of reactive oxygen species, and the induction of markers usually associated with programmed cell death, such as DNA fragmentation and metacaspase expression. This hypersensitive response was observed in

ten algal species belonging to four different orders, demonstrating its broad conservation among brown algae (Phaeophyta). Furthermore, TEM and *in vivo* staining assays suggest that the induction of algal autophagy might be another line of defence against *A. ectocarpii* and possibly, *Eu. dicksonii*. Pilot investigation of the heritability of resistance against *Eu. dicksonii* in *Ectocarpus fasciculatus* is consistent with the hypothesis that resistance is a phenotypically stable, quantitatively inherited trait. Our current efforts focus on the development of high-throughput phenotyping bioassays to identify the loci underpinning the resistance of the brown algae *Ectocarpus* and *Saccharina* against these pathogens, with the long-term view of breeding disease-resistant kelps for aquaculture purposes.

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PHYSIOLOGICAL PLASTICITY AND LOCAL ADAPTATION TO OCEAN ACIDIFICATION IN A CALCAREOUS ALGAE: AN ONTOGENETIC AND GEOGRAPHIC APPROACH

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To project how ocean acidification will impact biological communities in the future, it is critical to understand the potential for local adaptation and the physiological plasticity of marine organisms throughout their entire life cycle, as some stages may be more vulnerable than others. Coralline algae are ecosystem engineers that play significant functional roles in oceans worldwide and are considered vulnerable to ocean acidification. Using different stages of coralline algae, we tested the hypothesis that populations living in environments with higher environmental variability and exposed to higher levels of pCO₂ would be less affected by high pCO₂ than populations from a more stable environment experiencing lower levels of pCO₂. Our results show that spores are less sensitive to elevated pCO₂ than adults. Spore growth and mortality were not affected by pCO₂ level; however, elevated pCO₂ negatively impacted the physiology and growth rates of adults, with stronger effects in populations that experienced both lower levels of pCO₂ and lower variability in carbonate chemistry, suggesting local adaptation. Differences in physiological

plasticity and the potential for adaptation could have important implications for the ecological and evolutionary responses of coralline algae to future environmental changes.

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SCYTONEMIN BIOSYNTHESIS IN A NON-CULTURABLE CYANOBACTERIUM WITHIN HALITE ROCKS IN RESPONSE TO UV-A RADIATION

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Cyanobacteria (previously known as blue-green algae) are unicellular or filamentous Gram-negative, photoautotrophic bacteria that carry out oxygenic photosynthesis. They colonize a large variety of habitats including halites (evaporitic rocks with 95–99% NaCl) in Salar Grande, a coastal saltflat in the Atacama Desert, Chile. A halotolerant, unicellular cyanobacterium from the *Halotheca* genus is the dominant phototrophic microorganism inhabiting that substratum. The substratum provides transparency to photosynthetically active radiation and protection against solar UV light. Cyanobacteria are known to synthesize the pigment scytonemin as a UV resistance mechanism to avoid photochemical damage even when cells are subjected to long periods of desiccation. Scytonemin is a stable, lipid soluble heterocyclic dimer, localized at the extracellular sheath of cyanobacteria, that absorbs UV-A light (315–400 nm) with an *in vivo* absorption maximum at 384 nm. An 18-genes cluster associated with scytonemin biosynthesis was shown to co-transcribe in the cyanobacterium *Nostoc punctiforme* (ATCC 29133) exposed to UV-A irradiation. The objective of this work was to evaluate whether one key gene of the scytonemin biosynthetic pathway in cyanobacteria, *scyB*, was actively expressed in cells endolithically colonizing halite rocks. This work provides the first experimental design to study *in situ* gene expression in halite microbial communities. Experiments

were designed to irradiate halite rock fragments at different doses of UV-A light and to measure the relative transcription level of the *scyB* gene using quantitative RT-PCR. We found a 1.7-fold increase in the expression of *scyB* after 48 h of continuous UV-A irradiation. This is the first evidence for scytonemin biosynthesis in a non-culturable cyanobacterium within halite rocks and it will contribute knowledge about the mechanisms of resistance of this unique cyanobacterium to natural UV exposures. This work is also the first step in evaluating *Halotheca* as a source of scytonemin for future applications in pharmaceutical or biotechnological applications.

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A NOVEL TREATMENT PROTECTS CHLORELLA AT COMMERCIAL SCALE FROM THE PREDATORY BACTERIUM VAMPIROVIBRIO CHLORELLAVORUS

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The predatory bacterium, *Vampirovibrio chlorellavorus*, can destroy a *Chlorella* culture in just a few days, rendering an otherwise robust algal crop into a discolored suspension of empty cell walls. *Chlorella* is used as a benchmark for open pond cultivation due to its fast growth. In nature, *V. chlorellavorus* plays an ecological role by controlling this widespread terrestrial and freshwater microalga, but it can have a devastating effect when it attacks large commercial ponds. We discovered that *V. chlorellavorus* was associated with the collapse of four pilot commercial-scale (130,000 L volume) open pond reactors. Routine microscopy revealed the distinctive pattern of *V. chlorellavorus* attachment to the algal cells, followed by algal cell clumping, culture discoloration and ultimately, growth decline. The “crash” of the algal culture coincided with increasing proportions of 16s rRNA sequencing reads assigned to *V. chlorellavorus*. We designed a qPCR assay to predict an impending culture crash and developed a novel treatment to control the bacterium. We found that (1) *Chlorella* growth was not affected by a 15 min exposure to pH 3.5 in the presence of 0.5 g/L acetate, when titrated with hydrochloric acid and (2) this treatment had a bactericidal effect on the culture (2-log decrease in aerobic counts). Therefore, when qPCR results indicated a rise in *V. chlorellavorus* amplicons, we found that the pH-shock treatment prevented the culture

crash and doubled the productive longevity of the culture. Furthermore, the treatment could be repeatedly applied to the same culture, at the beginning of at least two sequential batch cycles. In this case, the treatment was applied preventively, further increasing the longevity of the open pond culture. In summary, the treatment reversed the infection of *V. chlorellavorus* as confirmed by observations of bacterial attachment to Chlorella cells and by detection of *V. chlorellavorus* by 16s rRNA sequencing and qPCR assay. The pH-shock treatment is highly selective against prokaryotes, and it is a cost-effective treatment that can be used throughout the scale up and production process. To our knowledge, the treatment described here is the first effective control of *V. chlorellavorus* and will be an important tool for the microalgal industry and biofuel research.

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ENGINEERING ACETIC ACID TOXICITY TO GROW MIXOTROPHIC MICROALGAE AT SCALE

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The benefits of combining heterotrophic and photoautotrophic metabolism to grow mixotrophic microalgae (i.e. productivities, titers) are well documented at a small scale, but such an approach has rarely been applied commercially. Microbial contamination is an obstacle to scaling up mixotrophic processes because the sugars used in such medium dramatically broadens the range of bacterial competition to which the reactor is exposed. Therefore, growth restrictive conditions that favour algal growth are required to exploit mixotrophy commercially. We screened several organic substrates with the green alga *Micractinium inermum* and observed that acetic acid conferred a competitive advantage over a wide range of bacterial contaminants. This led us to investigate the mechanisms of acetic acid toxicity in microalgae. We hypothesized that the acetate ion, rather than the acid form, is ultimately responsible for its toxicity and built a mathematical model that predicts the toxicity level in an algal culture. The physiological assumptions of the model were experimentally validated. Bacterial and algal responses to acetate toxicity were measured and used to design a mixotrophic process in which acetate acts both as a substrate and a contamination controlling agent. This

contamination control approach has led to what we believe is the first scale up of an industrial mixotrophic process.

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CHANGES OF SEDIMENTARY DIATOMS AND RESTING SPORES OVER A 60 YEAR PERIOD IN THE EAST CHINA SEA AND THEIR ENVIRONMENTAL INDICATION

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On a timescale of several decades, both climate change and anthropogenic activities have significantly affected the ecosystem of East China Sea. Diatoms, as an important constituent of phytoplankton, are good biological indicators of aquatic environmental changes. In the present study, a 53 cm-long sediment core was collected in the East China Sea at 28°25'59.9"N, 122°0'29.9"E in 25 m water depth and the chlorophyll a, diatom assemblages and diatom resting spores of every 1 cm section of core samples were investigated. In total, 138 diatom species and 32 genera were identified from the samples. The dominant diatom species were *Skeletonema costatum*, *Melosira sulcata*, *Thalassionema nitzschioides*, *Thalassiothrix frauenfeldii*, *Cyclotella striata*, *Hyalodiscus radiatus*, *Pseudo-nitzschia multiseriata*, *Coscinodiscus decrescens* and *Actinopterychus undulates*. The dominant diatom genera were *Skeletonema*, *Thalassiosira*, *Melosira*, *Chaetoceros*, *Coscinodiscus*, *Thalassionema*, *Thalassiothrix*, *Cyclotella*, *Hyalodiscus*, *Pseudo-nitzschia*, and *Actinopterychus*. In general, an increase over a 60 years period (1951–2010) was shown for: the concentration of chlorophyll a, diatom abundance, numbers of diatom species and genera and the percentage of nanoplanktonic diatoms such as the genera *Thalassiosira* and *Skeletonema*. However, the relative abundance of diatom resting spores showed a different pattern of change, with higher values (about 45%) in 1983 and in 1960, and no diatom resting spores were detected from 1966 to 1977. The great increase of diatom abundance from

around 1995 to 2010 could be attributed to the higher percentage contribution of dominant nano-planktonic diatoms in the genera *Thalassiosira* and *Skeletonema*. The fact that the chlorophyll a, and the abundance and species numbers of diatoms increased as a general trend from 1951 to 2010 indicates the eutrophication and environmental changes in the East China Sea.

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REPRODUCTIVE EFFORT AND FROND ARCHITECTURE- UNDERSTANDING THE IMPACT OF HARVESTING ASCOPHYLLUM NODOSUM IN EASTERN CANADA

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The commercial harvest of *Ascophyllum nodosum* in eastern Canada is managed as a single species resource in which harvesting removes up to 25% of the standing biomass. Here we examine reproductive effort (RE) from both harvested and non-harvested sites across Nova Scotia to evaluate total contributions of frond reproductive biomass in relation to the vegetative biomass. By counting the number of receptacle initials formed during summer, and monitoring their changing number and biomass until maturation in the following spring, we show that previous estimates of RE do not account for extensive receptacle loss prior to maturation, particularly over the January to June period. Using this approach, we show that RE exceeded 100% of the vegetative biomass in most populations across both harvested and non-harvested sites. Our data demonstrate a lack of synchrony in reproductive maturation among fronds and populations, and show that single samples of reproductive biomass at assumed peak reproduction result in inadequate measures of RE. We also determined the distribution of vegetative and reproductive biomass, and number of receptacles along frond length during summer. We found that 65% of vegetative the biomass, 80% of receptacle number, and 85% of reproductive biomass occurred in the upper 50% of frond length, with less than 10% of these metrics

in the lower one quarter of the *Ascophyllum* frond. These results suggest that the commercial harvest carried out in the summer when receptacle initials are a minimal component of total frond biomass is having a major impact on the reproductive capacity of populations.

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BIOPROSPECTING FOR THE RIGHT CYANOBACTERIA IN ECOLOGICAL ARID SOIL RESTORATION

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Biological soil crust communities, the soil skin of arid lands, provide important ecosystem services, particularly regarding soil fertility and stability against erosion. In North America, and in many other areas of the globe, increasingly intense human activities, ranging from cattle grazing to military training, have resulted in the significant deterioration of biological soil surface cover of soils. With the intent of attaining sustainable land use practices, we are conducting a 5-year, multi-institutional research effort to develop feasible soil crust restoration strategies for US military lands. We are including field sites of varying climatic regions (warm and cold deserts, in the Chihuahuan Desert and in the Great Basin, respectively) and varying edaphic characteristics (sandy and silty soils in each). To enable this effort, we have successfully established “biocrust nurseries” that produce viable and pedigreed inoculum, as a supply center for biocrust restoration, and for research and development. We report on significant advances made on optimizing methodologies for the large-scale supply of inoculum based on a) pedigreed laboratory cultures that match the microbial community structure of the original sites, and b) “in soil” biomass enhancement, whereby small amounts of local crusts are nursed under greenhouse conditions to yield hundred-fold increases in biomass without altering significantly community structure. Given the proven presence of cyanobacteria biogeographic patterns of distribution, bioprospecting for the right community structure with phylotypes matched to the local flora not only ensures maximum inoculum adaptability, but also prevents accidental cross-contamination with potentially invasive strains. It should become a standard in restoration efforts.

BACTERIA-MICROALGAE INTERACTIONS THROUGH NITROGEN AND COBALAMIN (B₁₂): FROM COMPETITION TO MUTUALISM

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Interactions between photosynthetic and non-photosynthetic micro-organisms play an essential role in natural aquatic environments and in microalgae cultures. These interactions include especially trophic relationships, organic matter remineralization, cell signalization and vitamins production. We characterized hundred bacterial strains isolated from our algal collection and selected a strain of *Alteromonas* sp. that promoted the growth of microalgae (*Dunaliella* sp.) in batch cultures by remineralization of organic nitrogen (Le Chevanton et al., 2013). Studies in chemostat suggested that the interactions switched from mutualism to competition for mineral nitrogen depending on the level of nitrogen limitation and on the bacteria/algae ratio. From our study we suggest that competitive or mutualistic relationship between microalgae and bacteria largely depends on the ecophysiological status of both microorganisms (Le Chevanton et al., 2016).

We recently focused our studies on the interactions through vitamin B₁₂ spectrum (Charlotte Nef thesis, 2016-2019). B₁₂ is a growth-promoting cofactor produced by archaea and bacteria. 50% of microalgae species are known to be auxotrophic for vitamin B₁₂. We tested bacterial consortia able to sustain the growth of the B₁₂ auxotrophic model haptophyte *Tisochrysis lutea*. A first consortium was selected but the B₁₂ production by bacteria was not able to fulfill the entire algae needs. On the basis of our own B₁₂ assays on *T. lutea* and on published data on the yields of bacterial B₁₂ production (Croft et al., 2005) and algal needs (Droop, 1957), we estimated the amounts of bacteria necessary to support the optimal growth of algae. Our results indicated that a minimal ratio above 100 bacterial cells per algae is needed to support the optimal growth for *T. lutea*. These results will be confirmed by experimental approach but already give away numerous questions including potential

competition for mineral nitrogen, sources of organic carbon for bacterial growth, B₁₂ secretion etc...

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USE OF MUTANT STRAIN, ECOPHYSIOLOGICAL STUDIES, AND DEEP PROTEOMIC ANALYSIS REVEAL MECHANISMS OF NITROGEN ACCLIMATION AND CARBON PARTITIONING IN THE HAPTOPHYTE *TISOCHRYSIS LUTEA*

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Haptophytes are a diverse monophyletic group with a worldwide distribution, and are known to be significantly involved in global climate regulation. Because nitrogen is a major limiting macronutrient for phytoplankton in oceans and for cultures of microalgae, understanding the involvement of nitrogen availability in haptophyte carbon partitioning is of global and biotechnological importance. The haptophyte *Tisochrysis lutea* is traditionally grown for aquaculture feed and it presents biotechnological potential for health and energy. For a few years, our lab has been accumulating ecophysiological and molecular data including transcriptomics and genomics. Here, we made an ecophysiological study, coupled with comprehensive large scale proteomic analysis, to examine differences of behavior in reaction to nitrogen availability changes between a wild type strain of *Tisochrysis lutea* (WTc1) and a mutant strain

(2Xc1) known to accumulate more storage lipids. Strains were grown in chemostats and studied under different eco-physiological conditions including N limitation, N repletion and N depletion. Whereas short term N repletion triggered consumption of carbohydrates in both strains, storage lipid degradation and accumulation during changes of eco-physiological status were only recorded in 2Xc1 but not in WTc1. After 3 months of continuous culture, 2Xc1 exhibited an unexpected increase in carbon sequestration ability (+50%) by producing twofold more carbohydrates for the same nitrogen availability. Deep proteomic analysis by LC-MS/MS identified and compared the abundance of 4,332 proteins, i.e. 25% of the genes. Results suggest that signalling mechanisms are involved in the upper sensibility to nitrogen changes, and mechanisms of DNA maintenance could be involved in the change in carbon accumulation in the mutant strain. Moreover, storage lipid accumulation seems to be favored by an overall reorganization of carbon partitioning in 2X cells that increases the metabolism of carbon and energy acquisition, and decreases mitochondrial activity and metabolic conversion of storage lipids to phosphoenolpyruvate. These results give new insights on the metabolism of the model haptophyte *T. lutea*.

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PHYLOGENETIC CHARACTERISATION OF OOMYCETES INFECTING TOXIC SPECIES OF THE MARINE DIATOM PSEUDO-NITZSCHIA

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In all ecosystems, parasites of primary producers can influence the 'whole community composition' and thus affect downstream ecological processes. Marine plankton sampling problems, the limited number of cultivable species, and the dearth of morphological information about

microalgae parasites hinders the assessment of their diversity, phylogenetic position and ecological importance. Using morphological observations, a parasite of the marine toxigenic diatom *Pseudo-nitzschia pungens* has been tentatively affiliated to the oomycete genus *Ectrogella*, which has been reported to infect both marine and freshwater diatoms. No phylogenetic affiliation was obtained, opening the debate about its systematic position. By single-cell genetic analyses, 18s-rDNA sequences of six distinct intracellular eukaryotic parasites were obtained, infecting four toxic *Pseudo-nitzschia* and one *Melosira* species on the North Atlantic coast.

Robust phylogenetic analyses demonstrate that these sequences cluster into two separate clades within the phylum Oomycota, relating to the seaweed parasite genera *Anisolpidium* and *Olpidiopsis*. Morphological features were insufficient to unambiguously attribute these parasites to any *Ectrogella* species. These two Oomycota clades have been named OOM_1 and OOM_2 awaiting further morphological and molecular information. A screening of global databases of the regions V4 and V9 of the 18s-rDNA, demonstrated the presence of these parasites beyond the North Atlantic coastal region. In a biweekly metabarcoding survey of the diatom communities in the Concarneau Bay (France, Brittany), high abundances of OOM_2 coincided once with the decline of *Pseudo-nitzschia* spp. and then with that of *Cerataulina pelagica*. This finding, together with the genetic identification of the same oomycete infecting both *Pseudo-nitzschia* australis and *Melosira* sp. supports the hypothesis of a lack of host specificity of the studied parasites.

These data highlight a complex and still unexplored genetic diversity within oomycete parasites of diatoms and calls for new biological evidence to unveil the evolutionary history and ecological role of these marine protists.

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STUDY OF TERRESTRIAL COELASTRELLA/ SCOTIELLOPSIS BIODIVERSITY IN THE SOUTH URAL REGION (RUSSIA)

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Despite the frequent discovery of *Coelastrella* Chodat and *Scotiellopsis* Vinatzer representatives in terrestrial

ecosystems, the true biodiversity of the genera in these habitats is uncertain. The aim of our study was to analyze the morphological and genetic diversity of *Coelastrrellal Scotiellopsis* species in soils of the South Ural region. Fifteen strains, isolated from different localities in boreal-forest and steppes zones, were investigated by DIC light microscopy and their diversity was assessed by ITS2 rDNA sequence comparisons. All strains could be divided into two morphotypes: a morphotype with visible meridional ribes and a smooth-celled one. A number of ribotypes were identified in these strains and all showed affinity to *Coelastrrellal Scotiellopsis* lineage. Only three ribotypes were identical or highly similar to authentic strains of *Coelastrrella corcontica* (Kalina & Puncochárová) Hegewald & Hanagata (P10), *Coelastrrella aeroterrestica* Tschalkner, Gärtner & Kofler (S8) and *Coelastrrella rubescens* (Vinatzer) Kaufnerová & Eliás (S24). The remaining nine ribotypes were close to known isolates of *Coelastrrellal Scotiellopsis* but cannot be related to any morphospecies.

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THE EFFECT OF REMOVING CARBON LIMITATION ON DIATOM AGGREGATION AND PHYSIOLOGICAL RESPONSES WHEN EXPOSED TO OIL

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Ocean acidification could reduce the stress of carbon limitation on silicifying diatoms which play a key role in the marine carbon cycle. Further, these phytoplankton are key contributors to marine snow formation through the production of exopolymeric substances (EPS). Diatoms are also thought to produce EPS as a means of protection from harmful substances, such as spilled oil. While little is known about the effects of oil spills on phytoplankton, even less has been studied about the potential future implications of these events under lower pH conditions. Using *Thalassiosira pseudonana*, a small centric diatom, we

conducted a series of roller tank experiments to mimic deep water processes. Four treatments including a control, enhanced $p\text{CO}_2$ levels, a water accommodated fraction (WAF) of oil, and enhanced $p\text{CO}_2$ with WAF. We found treatment specific marine snow as well as marine oil snow (MOS) formation with larger aggregates forming under elevated $p\text{CO}_2$ conditions compared to controls and irregular morphologies when WAF was present. Photosynthetic efficiency (using Fv/Fm as a proxy) was lowered in the WAF treatments compared to control and $p\text{CO}_2$. Similar such alterations to the photosynthetic apparatus (using sigmaPSII as a proxy) were also observed. The differences in aggregate morphology across treatments shows that further study is necessary to examine their composition and the factors which are important in driving their formation. The greater loss of oil and cells to aggregates (MOS) in ambient conditions suggests that *T. pseudonana* may be less efficient in future oceans, especially in polluted waters.

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RELATIONSHIPS BETWEEN PHOTOSYNTHETIC EUKARYOTES AND NITROGEN-FIXING BACTERIA OFF BRAZIL

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Symbioses between eukaryotic algae and nitrogen-fixing bacteria have been recognized in recent years as a key source of new nitrogen in the oceans. Using a combination of flow cytometry sorting and high throughput sequencing of the 18S rRNA gene V4 region and *nifH* we have investigated the composition of the photosynthetic pico and nano-eukaryote communities in conjunction with their associated nitrogen-fixing bacteria in the Brazilian South Atlantic Bight. Two distinct types of communities are most often encountered, one dominated by the Mamiellophyceae *Bathycoccus* and *Ostreococcus*, and one dominated by a prymnesiophyte known to be symbiotic with the UCYN-A1 cyanobacterium. Indeed, the UCYN-A1 *nifH* sequence is the one most often encountered. However we also found sequences from three other UCYN-A clades (A2 to A4),

with A2 being clearly associated to *Braarudosphaera bigelowii*. We also found an abundant nifH sequence distantly related to *Rhizobium* but previously found in the marine environment, including off Brazil, that could constitute a new symbiotic association with oceanic phytoplankton.

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THE CROSS-KINGDOM INTERACTION IN THE MARINE MACROALGAE *ULVA*: REVISITING THE LOTTERY THEORY THROUGH CROSS-TESTING OF BACTERIA

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Green macroalgae, mainly represented by the Ulvophyceae, constitute key primary producers of estuaries and coastal marine ecosystems. *Ulva* is also an important parameter in coastal ecosystem management as it can form massive nuisance blooms (“green tides”) in shallow environments. For a deep understanding of *Ulva* species development, morphogenesis, life-cycle regulation and life-history strategies, controlled laboratory-based culture of these algae is required. *Ulva* species, like other macroalgae, harbour a rich diversity of epiphytic bacteria that promote growth and morphological development. In the absence of bacteria, algal germ cells develop into colonies consisting of undifferentiated cells with abnormal cell walls. A breakthrough in understanding how bacteria control *Ulva* development was the establishment of a standardized tripartite community consisting of *Ulva mutabilis* (Føyn) and just two isolated bacterial strains, *Roseovarius* sp. strain MS2 and *Maribacter* sp. strain MS6. Employing this standardised tripartite model system, our research aims to determine the specificity of bacteria-induced morphogenesis of *Ulva* by the cross-testing of two very well investigated *Ulva* species, the emerging model system *U. mutabilis* and the cosmopolitan and economically important species *U. intestinalis*. Specifically, the survey revealed that pairs of bacterial strains isolated from other *Ulva* sources than *U. mutabilis* and *U. intestinalis* can completely recover growth and morphogenesis of *U. mutabilis* or *U. intestinalis* gametes under axenic conditions. This study also highlights that different compositions of bacterial guilds with similar functional characteristics can enable complete algal development and thus supports the “competitive lottery” theory, implying

that assemblage of bacterial population on a specific niche (algal surface) might be random and based on presence of functional genes rather than belonging to particular taxonomic entities.

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ENVIRONMENTAL PERTURBATIONS AND CELL COMPOSITION

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Environmental perturbations are tackled by algal cells in a variety of modes that are a function of the intensity of the perturbation, its impact on metabolic processes, its duration and the growth rate.

In response to perturbation, complex acclimation response can occur. Under certain circumstances, e.g. when the reproductive advantage afforded by acclimation is limited, homeostasis can be maintained.

In this talk, I shall try to provide a panorama of the various options and describe their consequences on the physiology and the composition of microalgae.

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BIO-FOULING IN CLOSED PHOTOBIOREACTOR SYSTEMS DURING MICROALGAE CULTIVATION AS CONSEQUENCE OF ITS ASSOCIATED MICROBIOME

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Several production systems have been designed for the purpose of industrial microalgae cultivation. The ProviAPT is one of the most promising due to its novel configuration. It includes an array of vertical flat-panels enclosed

in a translucent plastic bag filled with water. This feature increases productivity compared to other systems. Major bottlenecks at the industrial scale are bio-fouling and biofilm formation. These biofilms are formed on the reactor surface due to interactions between algae and other microorganisms present in the system. Consequently, the light penetration is reduced over time and productivity is diminished.

The Marie Skłodowska-Curie Initial Training Network ALFF is a European consortium whose central objective is the study of the algal microbiome. This particular project belongs to this consortium and aims to understand the microbial interactions that induce biofilm formation in photobioreactors, using the ProviAPT system as a model.

This work studies the microbial population of biofilms at different stages during *Nannochloropsis* sp. production. The samples consist of algae broth from reactors with different productivities and biofilms at different stages. From these samples, DNA was extracted, amplified on the V1-V3 hypervariable region of the 16S rDNA and the V4 region of the 18S rDNA and sequenced. From the same samples, microbial isolation on agar plates was performed. A total of 33 different bacterial strains were isolated and identified based on Sanger sequencing on the 16S. All bacterial isolates were individually tested on a biofilm formation assay. This assay reported potential candidates that could be the main agents inducing biofilm formation on the ProviAPT reactors during industrial microalgae cultivation. Thus, these microorganisms are current targets in order to design a biofilm prevention strategy.

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MICROCOLEUS VAGINATUS CARRIES A NITROGEN-FIXING MICROBIOME THAT CAN HELP IT COLONIZE NUTRIENT-DEFICIENT ARID SUBSTRATES

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Biological soil crust (biocrusts) communities provide important ecosystem services to arid lands regarding soil fertility and stability, and are arguably the most extensive biofilm on Earth. They constitute a carbon pool that exceeds 1014 g C, and are responsible for almost half of the nitrogen fixed on land. The cyanobacterium *Microcoleus vaginatus* is the pioneer of biocrust communities, but interestingly this architect of the early biocrust successional stage does not fix nitrogen. Where does the initial nitrogen pulse come from to support the establishment of *M. vaginatus* as it colonizes bare soil? To answer this question, we compared the bacterial community firmly attached to *M. vaginatus* bundles (the “cyanosphere”) to that of the bulk biocrust soil, using high throughput 16S rDNA gene sequencing. We found a distinct bacterial community that is significantly enriched in the cyanosphere of *M. vaginatus*, one that contains several of the recently identified heterotrophic biocrusts nitrogen fixers. We hypothesized that nitrogen fixing heterotrophs could be differentially abundant in this community. Using real-time PCR we demonstrated that the *nifH* genes were 100 fold more abundant in the cyanosphere than in the rest of the soil. In conjunction with recent metabolomics studies, this strongly suggests a symbiotic mechanism by which *M. vaginatus* provides carbon to the heterotrophic community in its cyanosphere and in exchange, this community provides *M. vaginatus* with fixed-nitrogen. This study reveals the existence of a differentiated microbial community associated with *M. vaginatus* and proposes a symbiosis with its cyanosphere that could be key to the early establishment of the biocrust.

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NEW AND INTERESTING SPECIES FROM THE GENUS LUTICOLA (BACILLARIOPHYCEAE) IN WATERBODIES OF SOUTHEASTERN ASIA

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Several *Luticola* species are found in different water ecosystems of Laos, Cambodia and Vietnam. Of those described

previously we have recorded *L. belawanensis* Levkov & Metzeltin, *L. tropica* Levkov, Metzeltin & Pavlov, *L. inserta* (Hustedt) D.G. Mann, *L. intermedia* (Hustedt) Levkov, Metzeltin & Pavlov, *L. tujii* Levkov, Metzeltin & Pavlov and *L. taylorii* Levkov, Metzeltin & Pavlov. Description of morphology and measurements of these species are presented and compared with previous records. Five species new to science were found: *L. laosica* Glushchenko, Kulikovskiy & Kociolek sp. nov., *L. bolavenensis* Glushchenko, Kulikovskiy & Kociolek sp. nov., *L. robustaformis* Glushchenko, Kulikovskiy & Kociolek sp. nov., *L. renelecohui* Glushchenko, Kulikovskiy & Kociolek sp. nov. and *L. pseudodistinguenda* Glushchenko, Kulikovskiy & Kociolek sp. nov. New species are illustrated by LM and SEM micrographs. Distribution of Lenticularia species from Southeast Asia are discussed.

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CHARACTERIZATION OF THE EPIPHYTIC BACTERIAL COMMUNITY ASSOCIATED WITH THE KELP LAMINARIA DIGITATA

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Numerous studies on epiphytic microbes of macroalgae have highlighted their important role in algal health and physiology. These epiphytic microbes may allow the host to adapt to varying environmental conditions, induce host morphogenesis, or protect the host's surface from harmful colonization. This microbiota is also affected by interactions with the alga. The kelp *Laminaria digitata* possesses

a unique defence metabolism, associated with the production of toxic iodine compounds which may control its surface biofilm and/or repulse microbial pathogens. Therefore, bacteria adapted to these particular living conditions probably possess genetic material adapted to the metabolism of iodine. Also, *L. digitata* gradually concentrates iodine in its tissues depending on age, thallus areas, and season. Subsequently, the associated epiphytic microbial community is expected to vary along the thallus according to these characteristics. We thus propose (i) to identify the bacterial community associated with *L. digitata* according to different regions along the thallus and different maturation stages of the alga and (ii) to develop a comparative genomic approach for characterizing important functions of some cultivable bacteria isolated from *L. digitata*. Plantlets, blade, and stipe samples from four wild adult sporophytes of *L. digitata* were collected on the shore, and the taxonomic composition and the structure of their associated epiphytic microbiota were determined using metabarcoding. As shown by multivariate analyses, plantlet microbiota was more similar to blade microbiota and the four stipe samples strongly clustered together, showing a clear structuring of the epiphytic community of *L. digitata* according to tissues or age of the alga. Further, sequenced genomes of bacteria isolated from different areas of *L. digitata* should allow us to determine how these bacteria may be adapted to the metabolism of the algal host.

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EXPLORING HUMAN-INDUCED EVOLUTION USING GENOMICS OF REVIVED DIATOMS FROM NATURAL ARCHIVES

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We are using resting stages of the common diatom *Skeletonema marinoi* revived from sediment cores to study the mechanisms of adaptation in nature, through the integrated assessment of experimental tests of trait adaptation and genotypic evolution. A rich bed of resting propagules, priority effects and local adaptation provide strong genetic differentiation and allow single populations to monopolize local sites. From isotope-dated sediment in Scandinavian fjords, with bottom anoxia, we have germinated populations from the past and compared their respective genomes and phenotypes with the present populations. This promising system is used to evaluate evolvability over the past 100 years, which in this organism represent 40,000 mitotic generations. In this setting we have investigated potential adaptation to anthropogenic driven marine eutrophication. From populations of different ages in parallel fjords we have traced changes in phenotypic growth response to different levels of light and nutrition and have uncovered a clear adaptive trend. We have detected >17,000 SNPs in 100 candidate genes and their respective flanking regions. Subsequently, the phenotypic trends have been correlated to changes in SNP allele frequencies over time.

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TAXONOMY AND PHYLOGENY OF THE GENUS GAYLIELLA (CERAMIALES, RHODOPHYTA)

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Phylogenetic analyses and morphological data were used to segregate *Gayliella* from *Ceramium*, revealing a monophyletic genus in the tribe Ceramieae, distinct from other *Ceramium* species by having just one basipetal cell and unicellular rhizoids originating from periaxial cells. According to phylogenetic analyses, *Ceramium dawsonii* and *Ceramium*

flaccidum from Brazil belong to *Gayliella*. However, three separate haplotypes are seen in *C. dawsonii*, and Brazilian *C. flaccidum* does not fit the current circumscription of *Gayliella flaccida*, which occurs only in the North Atlantic Ocean. Since the establishment of the genus *Gayliella*, there have been no studies defining Brazilian species using molecular and morphological tools. This study aims to delimit *Gayliella* species along the Brazilian coast with molecular and morphological data. Towards this goal, specimens referred to *C. dawsonii*, *C. flaccidum* and twenty other samples from Brazilian coasts were evaluated based on three molecular markers (partial LSU, *rbcL* and *cox1*) and comparative morphology. Our data showed that Brazilian specimens were placed in five distinct well-supported lineages: one haplotype of *C. dawsonii* which is transferred to *Gayliella*; two other haplotypes of *C. dawsonii* which are now described as new species of *Gayliella*; and another new *Gayliella* species based on Brazilian "*C. flaccidum*". Our findings highlight the cryptic diversity in this group, and show the importance of molecular assisted alpha taxonomy in defining *Gayliella* species.

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CHANGES IN MACROALGAE COMMUNITIES DUE TO NATURAL CO₂ GRADIENTS

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Natural CO₂ vents are excellent laboratories in which to study the effects of ocean acidification (OA). Very recently, a new natural vent has been discovered in La Palma (Canary Island), with exceptional features to study the oceans of tomorrow, such as 7.6 pH average, shallow waters and no bubbles. The aim of this study was to characterize the biodiversity changes in benthic macroalgae communities along the natural pH gradient. To do this, we sampled four

sites at different distances from the vent center. At these sites, we scraped 20 × 20 quadrats of the rocky bottom and collected the enclosed assemblage. Then, the samples were separated in two fractions with 65 µm and 1mm sieves. Metabarcoding techniques were used to analyze mitochondria cytochrome c oxidase, subunit I (COI) marker of each fraction of the collected samples. MDS ordination and ANOSIM test revealed significant changes in MOTUs (Molecular Operational Taxonomic Unit) composition between sites. We observed an increase in red algae and turf type communities as we got closer to the vent, with Rhodophyta orders the most relevant inside the vent. There was, however, an exception to this pattern, *Halopteris* sp. (order Sphacelariales of Ochrophyta) also characterized the vent site. Calcareous species were very limited at the vent site, but increased with distance from the vent. Finally, the species diversity of the small (65 µm) fraction increased near the vent. We demonstrate that OA can modify the taxonomic composition, the structure and algae species diversity in future oceans.

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CHARACTERIZATION OF THE CYTOSKELETON OF PORPHYRA UMBILICALIS AND COMPARISON TO OTHER RED ALGAE BASED ON ANALYSIS OF THE COMPLETED PORPHYRA GENOME

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The cytoskeleton is a dynamic network of protein filaments, molecular motors, and associated regulatory proteins that drives mitosis, cytokinesis, intracellular transport, and cell motility, and it participates in other processes including chromatin remodeling, signaling, and cell polarity. Thus, characterization of an organism's cytoskeleton provides insight into many aspects of its biology. Consequently, the cytoskeleton is well-studied in many organisms including in plants and animals; however, the red algal cytoskeleton remains poorly understood. Using bioinformatic tools including BLAST and PSI-BLAST, we searched the recently completed *Porphyra* nuclear genome for key filament, motor, and regulatory proteins, and found that the complement of cytoskeletal components in *Porphyra* is significantly reduced relative to other multicellular organisms. For example, while the actin and tubulin filament proteins are

present, as expected, a number of key regulatory proteins are absent, and the number of motors is especially reduced. In particular, the actin motor myosin is entirely absent, and only a small set of kinesins and no dynein motors are present. These observations raise questions about how *Porphyra* cells accomplish intracellular transport. We have extended our cytoskeletal analyses to other red algae with sequenced genomes and find that the only universal motor proteins are a small number of kinesins. Another surprising observation is the lack of the actin nucleating complex ARP2/3, which has been believed to be essential for amoeboid motility, raising questions about how *Porphyra* neutral spores accomplish amoeboid movement. The array of nuclear actin-related proteins is also reduced, suggesting that *Porphyra* might have a reduced ability to regulate chromatin remodeling as compared to both green plants and animals. Overall, we suggest that the reduced cytoskeletal capabilities and complexity of *Porphyra* and other red algae may explain apparent limitations to cell size and the ability to form complex tissues in comparison to other multicellular organisms.

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EXPERIMENTAL RESEARCH ON LIPID ACCUMULATION IN MARINE DIATOM (BACILLARIOPHYTA) CELLS: INFLUENCE OF LIFE CYCLE, PHYSIOLOGY AND PHYLOGENY

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Diatoms (Bacillariophyta) are unicellular eukaryotic and photoautotrophic microorganisms of various shapes and they range in size from ca. 1 to 600 µm (rarely larger). Because of this small size and rapid reproduction rate, the biomass of diatoms in world oceans is very large. This makes diatoms important oxygen and biomass producers in the oceans, and they have a major role in global carbon and silica cycles in aquatic ecosystems. Due to their adaptation to a wide variety of conditions, simple unicellular structure and fast growth, diatoms are used as a tool in various disciplines such as water quality assessment or climate

paleoreconstructions. Recently, attention is focused on potentially valuable bioproducts extracted from the diatoms, such as pigments used in pharmacy and medicine or lipids for biofuel production. This project is focusing on several taxa representing one genus of diatoms to provide precise analysis on aspects of their biology. The purpose of this project is to investigate the monoraphid diatom genus *Schizostauron* Grunow, which has an uncertain taxonomic and phylogenetic position, in order to answer the following questions: (1) Is the amount of lipids accumulated in *Schizostauron* cells dependent on diatom size or life cycle? (2) Under which conditions is lipids accumulation greatest? (3) Is the ability to produce oil a heritable trait? (4) Is there any phylogenetic signal for oil-producing *Schizostauron* taxa?

To answer those questions, in this project the following analyses will be conducted: cell wall morphology analysis, molecular analysis of the available strains and observations of the life cycle and sexual reproduction pattern, which has already been successfully initiated in certain strains. All of these analyses are crucial to determine the taxonomic position of the genus and identify species, which will enable us to answer further questions concerning lipid accumulation.

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POPULATION GENOMIC OF UNDARIA PINNATIFIDA: INSIGHTS INTO MECHANISMS OF GENOME ADAPTATION DURING INVASION

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The genus *Undaria* regroups large multicellular algae commonly known as kelps. Within its natural range in Eastern Asia, species of the genus *Undaria* play an important ecological role. In Korea *Undaria pinnatifida* is extensively cultivated (~500,000 tons/year) as a food source and for its extracts. Unfortunately *Undaria pinnatifida* is also well known for being an invasive species. Indeed it was introduced to Europe in the 1970s associated with oysters

introduced for fisheries purposes, initially to the Mediterranean coast and later to Brittany. In the late 1980s the species was also recorded in New Zealand. Introduced populations of *Undaria pinnatifida* are considered to cause considerable impacts on coastal ecosystems by forming dense canopies, displacing native species, and thus reducing biodiversity. It also causes economic impacts on fishing and aquaculture.

To investigate the whole-genome adaptation of *Undaria pinnatifida* during invasions, we produced the draft genome of *Undaria pinnatifida* from Korea and resequenced the genomes of 22 individuals from various invasive populations around the world. We explored genome-wide variations between the different populations considered and notably, we identified putative selective sweeps at multiple loci. This study will provide new insights into the mechanisms by which genomes can rapidly adapt during an invasion. Furthermore, the genome sequence of *Undaria pinnatifida* and its polymorphism represent a major resource for future crop improvement and biotechnology.

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MODELLING THE SEASONAL GROWTH OF THE BROWN SEAWEED FUCUS VESICULOSUS IN THE KIEL OUTDOOR BENTHOCOSMS

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Warming and acidification of the oceans as a consequence of increasing CO₂-concentrations occur globally. In mesocosm experiments, the single and combined impact of elevated seawater temperature and pCO₂ (1,100 ppm) on the brown alga *Fucus vesiculosus* together with its associated community (epiphytes and mesograzers) was studied in four consecutive experiments (from April 2013 to April 2014). Based on these experiments, a numerical boxmodel simulating the seasonal growth of *F. vesiculosus* in the Kiel Outdoor Benthocosms (KOBs) was developed. Nitrogen and carbon cycling in the KOBs were considered and relevant physiological and ecological processes were implemented. To run simulations under present and global

change scenarios (e.g. warming, ocean acidification) the model was forced with atmospheric and hydrographic data of the Kiel fjord. DIN and DIC concentration in the water and *Fucus* growth as carbon and nitrogen increase were explicitly modelled. For instance, the following processes were implemented: (1) Storage of carbon and nitrogen assimilates by *Fucus*, leading to a temporal decoupling of assimilation and growth. (2) Shading effects of epiphytes. (3) Grazing by *Idotea*, *Gammarus* and *Littorina* on both *Fucus* and epiphytes, but with species-specific rates and preferences. At present, the model is a suitable scientific tool capable of integrating our knowledge about macroalgal processes, their growth and productivity in coastal areas. It further facilitates the communication of complex knowledge to lay persons. Ultimately, the development of a predictive model, which can be coupled to a 3D-high resolution western Baltic Sea model, is anticipated. This will allow observations on the consequences of global change for the wellbeing and distribution of *F. vesiculosus* in the western Baltic Sea. Understanding responses of macroalgae and of the associated community is important because changing global temperatures and elevated CO₂ may affect the ecological role of *Fucus* as primary producer, carbon sink, water purifier, and ecosystem engineer in the coastal ecosystem of the Baltic Sea.

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BALANCING PHOTOSYNTHESIS AND RESPIRATION INCREASES MICROALGAL BIOMASS PRODUCTIVITY DURING PHOTOHETEROTROPHY ON GLYCEROL

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Glycerol was used as an organic substrate to enhance the biomass production rate of a *Dactylococcus* microalga during photoheterotrophy, while simultaneously reducing the need for cell culture gas exchange. Photoheterotrophic cultivations were carried out at concentrations of 6, 30, and 150 mM glycerol in parallel with photoautotrophic and heterotrophic cultivations. The highest biomass productivity was with 30 mM glycerol, the concentration where net

oxygen exchange between the cells and the culture medium was minimized, thus implying a balance between respiration and photosynthesis and internal recycling of O₂ and CO₂.

The maximum specific growth rate and biomass productivity were increased by 43 ±9% and 108 ±16%, respectively, compared to the photoautotrophic cultivations. The net oxygen production rate could be modeled as a function of the light intensity, chlorophyll content, the photosynthetic efficiency as measured by PAM fluorometry, and the respiration rate. Glycerol addition decreased the cellular chlorophyll a content and the photosynthetic efficiency, but increased carbon fixation by respiration. These results show the addition of a waste product, e.g., glycerol from biodiesel, could be used as an algal bioprocess co-substrate to reduce or eliminate the aeration energy demand while simultaneously increasing biomass production.

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COPEPODAMIDES – KEY PLAYERS IN PLANKTON INTERACTION

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Copepods are the dominant members of zooplankton throughout the oceans. They release copepodamides, a group of polar signaling compounds, which have various effects on different microalgae. Here we elucidate the structure of two novel copepodamides, as well as their impact on toxin formation and colony size in three different microalgae species. Dose response experiments with copepodamides revealed increased toxicity of the dinoflagellate *Alexandrium minutum* and the diatom *Pseudo-nitzschia seriata*, as well as reduced chain length of the diatom *Skeletonema marinoi*. Differing sensitivity of the dinoflagellate and diatom species to copepodamides indicates that distinct perception pathways might be involved in signal perception.

We performed pharmacological tests with *Skeletonema marinoi* to further investigate which receptor class is involved in copepodamide sensing. Initial results indicate that for this species G-protein-coupled receptors (GPCRs) are involved in signal perception. Our future work will focus on

the exact identification of copepodamide receptors in *Skeletonema marinoi*.

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DINOFLAGELLATES' (MICROBIAL) FRIENDS AND THEIR FUNCTIONS

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Marine phytoplankton live amongst a complex community of microbial associates that can affect their growth, physiology and behaviour. But what the relative importance of these microbial effects are, in comparison to the many other bottom-up and top-down controls of primary production, remain poorly characterised. This is largely because interrogating microscale interactions in natural samples is very difficult. This has resulted in the majority of investigations using 'simple systems', such as laboratory cultures with simplified microbial communities. Nevertheless, such studies are now unravelling mechanisms by which phytoplankton and bacteria may interact. In this presentation, we report ongoing work with marine dinoflagellates and their bacterial associates. This work has highlighted that some dinoflagellates have an obligatory requirement for bacteria following periods of dormancy, but not always during vegetative growth. A bacterially-produced co-factor is suspected, but unconfirmed. This work, coupled with bacterial community analysis, identified *Marinobacter* – a genus of oil-degrading bacteria – to be ubiquitous associates of dinoflagellates and many other micro and macro-algae. Moreover, *Marinobacter* nearly always turn out to be beneficial in some way for the algal host. For example, production of photo-active iron-binding ligands has highlighted how bacteria may mediate enhanced iron uptake rates by phytoplankton. However, we believe *Marinobacter*'s importance extends beyond just producing siderophores, and one speculation is that they may represent a keystone microbial species in algal-associated communities.

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THE NUCLEAR GENOME OF PORPHYRA UMBILICALIS (BANGIOPHYCEAE, RHODOPHYTA), A COMMERCIALY HARVESTED REPRESENTATIVE OF THE ANCIENT BANGIOPHYTES

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Aquatic food webs, biogeochemical cycling, and human health are strongly influenced by plastid and nuclear genes from red algae due to the evolution of diatoms, dinoflagellates, apicomplexans, and haptophytes through secondary endosymbioses involving ancestral red algae. Moreover, the oldest taxonomically-resolved fossil (1.2 Ga) of a multicellular eukaryote belongs to the Bangiophyceae. Here, we present the haploid nuclear genome (87.7 Mbp) of *Porphyra umbilicalis* (Bangiophyceae, Rhodophyta), which has a high G + C-content, with protein-coding regions having a G + C-content averaging 72.9%. Prior to use of PacBio, sequencing reactions disproportionately recovered antibiotic-resistant bacterial contaminants with moderate G + C-content. An improved and highly contiguous (contig L50 = 189.9 kb) draft assembly was generated with whole genome shotgun (WGS) sequencing using the PacBio platform, with insertions and deletions corrected using Illumina WGS. Nearly 98% of sequenced transcripts (ESTs) were mapped to the genome assembly and a complete complement of genes encoding RNA polymerase subunits and other conserved informational proteins involved in transcription, translation, and DNA synthesis were found, suggesting that the genome is nearly complete. Genes typically have ~2 exons, implying abundant splicing for a red alga; however, only 235 alternative splice-forms were identified from expression data. This genome encodes the largest number of gene loci (13,125) among characterized red algal nuclear genomes and contains ~44% repeat sequences (largely LTR and DNA transposable elements).

Despite the preparation of clonal, unialgal blades in media containing antibiotics and purification of genomic DNA on cesium chloride gradients, ~50% of the scaffolds in the PacBio assembly did not belong to *Porphyra*. Existing metagenomic binning tools were unable to partition the *Porphyra* genome scaffolds, so a PCA-based method supported by heuristics was developed. This allowed confident separation of scaffolds belonging to the *Porphyra* genome from metagenomic scaffolds to assemble a high quality genome.

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MODEL SPECIES IN BROWN ALGAE: A REAPPRAISAL OF ECTOCARPUS SPECIES DIVERSITY USING BOTH CLASSICAL AND MASSIVE SEQUENCING

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The genus *Ectocarpus* (Ectocarpales, Phaeophyceae) contains filamentous algae widely distributed in marine and estuarine habitats of temperate regions in both hemispheres. While *E. siliculosus* has become a model organism for genomics and genetics of the brown macroalgae, accurate species delineation, distribution patterns and diversity for the genus *Ectocarpus* remain problematic. In a first study, we started by clarifying the number of cryptic species in this group by using two unlinked loci (the mitochondrial COI-5P and the nuclear ITS1) and an integrative approach associating barcode gap detection analyses with Bayesian species delimitation analyses and phylogenetic reconstructions. These first results showed the presence of at least 15 cryptic species and suggest the existence of incomplete lineage sorting or introgression between five of

them. Moreover, the use of a large amount of specimens (729 *Ectocarpus* collected mainly along the European and Chilean coasts) allowed us to characterize the phylogeographic patterns, range and depth distributions of these cryptic species. In a second part of our study, we selected 120 *Ectocarpus* unialgal strains previously identified at the species level using the COI and the ITS1 and obtained for these individuals hundreds of unlinked nuclear loci via RAD sequencing. Our Radseq results retrieved a pattern of phylogenetic relationship within the *Ectocarpus* subgroup *siliculosi* concordant with the ones recovered in previous studies (i.e. existence of a strongly supported monophyletic group including *E. crouaniorum*) but were also able to resolve some of the relationships between species part of the paraphyletic “*siliculosi*” assemblage. Moreover, occurrence of potential hybridization between closely related and sympatric species was suggested. The use of these various independent markers have allowed us to clarify the species diversity within the *Ectocarpus* subgroup *siliculosi* and also to suggest the possible existence of different levels of reproductive barriers within this species complex, possibly.

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CONTRASTING CYTOPLASMIC AND NUCLEAR PATTERNS OF GENTIC DIVERSITY TO STUDY THE RED ALGAE SPECIES COMPLEX MAZZAELLA LAMINARIOIDES

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Mazzaella laminarioides (Bory) is a common haploid-diploid red alga that forms dense beds. This alga has a wide distribution range, covering 3,500 km of the Chilean coast,

but is restricted to high rocky intertidal zones. Recently, the existence of three highly divergent genetic lineages was demonstrated for this taxon, and two cytoplasmic markers were used to determine that these lineages are distributed in strict parapatry. Newly developed polymorphic microsatellite loci that cross-amplify in all three cytoplasmic lineages were genotyped in 24 sites (i.e. eight sites within each lineage). Both markers reveal a sharp transition between a Northern and a Central species of *Mazzaella* located at 32°48'S and no traces of hybridization were detected in the localities flanking this area. Within this transition zone of less than five-kilometers large patches of *Mazzaella* are encountered on the rocky shore. On the other hand, the transition between the Central and Southern species of *Mazzaella* is located between the 37°S and 38°S and is characterized by a mosaic of sites occupied by one species or the other where low levels of hybridization were observed. Clear sign of introgression of a Southern mitochondria was detected in LOB, the southernmost locality where the nuclear Central genetic group is observed. Interestingly, all the transition zones between the Central and Southern species correspond to habitat discontinuity (i.e. sandy beaches). This result raises the question as to the role of sandy beaches in the maintenance of parapatry and to their role as a reproductive isolation barrier between the two most recently diverging species of *Mazzaella*.

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HOW FLUCTUATING LIGHT IMPACTS GROWTH, PHOTOSYNTHESIS AND RESPIRATION OF PHYTOPLANKTON COMMUNITIES

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Climate change influences the mixing conditions of water bodies and thus light availability for phytoplankton. Despite the potential impact of vertical mixing on phytoplankton dynamics and productivity, effects of fluctuating light on phytoplankton are not well understood. Algal groups and species have evolved different strategies regarding light utilization and may cope with fluctuating light by specific photo-adaptive responses. Therefore, in well-mixed systems, the light absorption of algae, pigment concentrations, photosynthesis, respiration, and as a result, growth

may be affected. Effects of fluctuating light on the growth-light relationship of phytoplankton have been investigated for decades, but mainly focusing on individual species grown under laboratory conditions. Our study is one of the very few attempts to understand light dependency of algal physiology under fluctuating light in semi-natural conditions. In a shallow light-limited system dominated by diatoms and cyanobacteria, we simulated different mixing conditions by computer-controlled vertical motion of lake water samples. Light absorption, pigment concentrations, photosynthesis, respiration and algal growth of a phytoplankton community were compared. Our results show that, phytoplankton incubated under fluctuating light had lower growth efficiency at limiting light and higher light-saturated growth than its counterpart incubated under non-dynamic light. Under fluctuating light, algae take advantage of short and regular saturating light peaks by an increase of the maximal electron transport rate combined with reduced respiration losses due to photo-inhibition avoidance.

By focusing on the relevant dynamics of a phytoplankton community under fluctuating light in the lake, our study contributes to better understand the effects of vertical mixing on algae development in wind-exposed light-limited waterbodies.

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DNA METABARCODING OF PHOTOTROPHIC EUGLENIDS FROM A SELECTED HYPEREUTROPHIC RESERVOIR BASED ON THE ANALYSIS OF V2 18S rDNA

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Rapid and exact species identification is important for biodiversity assessment. DNA barcoding was proven to be an efficient technique to overcome the difficulties with identification caused by morphological similarities between the species and the lack of sharp diagnostic features. Furthermore, this method enables a comprehensive assessment of species composition, which is nearly impossible based on microscopic methods. The suitable barcode sequence must contain enough information to differentiate unambiguously between closely related taxa. Hence, the knowledge about the biodiversity of euglenids is constantly being advanced, the variable regions, V2-V3, V2, V4 and V8 of nuclear rDNA had been highlighted as sufficient DNA barcodes for phototrophic euglenids.

Here, we present the aims of the project which will use the V2 18S rDNA region for the metabarcoding of phototrophic euglenids in environmental samples taken from a selected pond. The research will cover both morphological and molecular (next generation sequencing – Illumina) assessment of euglenid specimens. We will investigate whether the proposed DNA barcode can be successfully used for genera and species identification. Moreover, the monitoring of water physicochemical parameters will be carried out in order to enrich the knowledge about the ecology of phototrophic euglenids.

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MORPHOLOGICAL AND PHYLOGENETIC ANALYSIS OF CYLINDROCYSTIS REVEALS NEW SPECIES AND EXTRAORDINARY SPORES WITH IMPLICATIONS FOR OUR UNDERSTANDING OF FOSSIL ZYGNEMATOPHYCEAE

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Spores of Zygnematophyceae are important for the identification of both fossil and extant species. However, spores are rarely encountered in extant populations making it difficult to connect extant taxa with their fossil relatives. Spores are necessary to identify species in the polyphyletic genus *Cylindrocystis*. In order to better understand their diversity, we completed a comprehensive study of 24 strains of

Cylindrocystis. These were grown in controlled laboratory conditions and subjected to growth conditions that might induce production of zygospores. None of the strains were found to be heterothallic and few homothallic strains produced zygospores. Two strains produced spores of a character previously unknown for any species of *Cylindrocystis*. Phylogenetic analysis of two plastid-encoded genes confirmed that these strains were phylogenetically distinct and represent new species. One of these new species produces a spore very similar to *Transeauina glyptosperma* and *T. smithii*. This suggests that those fossils previously assigned to *Transeauina* or its relatives may in fact represent species only distantly related to the *Transeauina* lineage.

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THE PLANKTONIC VOC METABOLOME

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Marine-derived VOCs directly impact atmospheric chemistry and are involved in secondary aerosol formation and growth of newly formed particles that can become cloud condensation nuclei. VOCs are actively produced and cycled in marine ecosystems, but VOC contributions to carbon budgets are poorly constrained. Knowledge about VOC sources and the conditions that influence their accumulation in the surface ocean is needed to advance global chemical transport models for which the underlying source terms for VOC air-sea exchange are not known, and neither is the overall contribution of VOCs to the marine carbon cycle. We present a new approach that couples proton-transfer-reaction time-of-flight mass spectrometry (PTR-ToF/MS) with seawater incubation chambers to measure net rates of VOC production in natural seawater suspensions. During four field campaigns to the North Atlantic Ocean (Nov. 2015, May 2016, Sept. 2017, Feb. 2018), we are assessing biologically-mediated VOC production during different stages of the annual phytoplankton bloom. Results from the first two field campaigns show that VOC emissions (including acetaldehyde, acetonitrile, acetone, and methanol) were higher during the annual spring phytoplankton bloom maximum (May 2016) than in winter (Nov. 2015) during deep seasonal mixing. Net production of some VOCs, including isoprene, displayed

clear diel variability. Variations in VOC emissions across space and time are associated with plankton community composition and phytoplankton productivity. These field-based results are complimented with laboratory culture experiments to identify the VOC metabolomes of environmentally important marine plankton. For example, we recently measured the real time production of both methanethiol and dimethylsulfide from the globally abundant heterotrophic bacterium, *Pelagibacter*. Similarly, we show that the light-dependent VOC metabolomes of cyanobacteria, green algae, and diatoms are distinct from one another. This work will help resolve significant uncertainties associated with VOC identities, biological sources, and environmental variability.

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HOW TO INDUCE SEXUAL REPRODUCTION IN CENTRIC DIATOMS

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The reproductive strategy of diatoms includes asexual and sexual phases, but in many species, including the model centric diatom *Thalassiosira pseudonana*, sexual reproduction has never been observed. Furthermore, the environmental factors that trigger sexual reproduction in diatoms are not understood. Although genome sequences of a few diatoms are available, little is known about the molecular basis for sexual reproduction. We demonstrate that ammonium reliably induces the key sexual morphologies, including oogonia, auxospores, and spermatogonia, in two strains of *T. pseudonana*, *T. weissflogii*, and *Cyclotella cryptica*. RNA sequencing revealed 1,274 genes whose expression patterns changed when *T. pseudonana* was induced into sexual reproduction by ammonium. Some of the induced genes are linked to meiosis or encode flagellar structures of heterokont and cryptophyte algae. The *T. pseudonana* F1 generation is characterized by a 450% enlargement by volume over the parental generation and altered growth rate. The discovery that ammonia induces sex in centric diatoms opens avenues for investigation of diatom reproductive mechanisms and the roles sex plays in aquatic ecology.

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CHARACTERIZATION OF TWO BENTHIC DIATOMS ISOLATED FROM ORAN COAST

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Diatoms (Bacillariophyta) are photosynthetic unicellular organisms with silica cell walls (frustules) that constitute important primary producers in coastal waters. In Algeria few studies have been made on diatoms, but in 2010 Nehar et al. recorded 10 taxa from El-Hammam stream, a thermal water at Mascara, and the estuary of the Cheliff river at Mostaganem. In our study two strains of benthic diatoms were isolated from coastal water in Oran (Algeria) and maintained by resubculturing in controlled light (14:10 L: D, 75 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) and temperature conditions ($19 \pm 1^\circ\text{C}$).

Strains were identified as *Entomoneis alata* and *Nitzschia* sp. by optical and scanning electron microscopy. Growth rates, total extractable lipid content and chlorophyll a concentration were compared when cultured under different light intensities (10, 25, 60, 100 and 300 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$). Results showed that both biomass and lipid production were higher under lower incident irradiance of 10 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ and 25 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ and ranges of lipid production varied from 50 to 600 mg L⁻¹. At 60 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, *Nitzschia* sp. produced the maximum concentration of chlorophyll a, 2.88 pg cell⁻¹ whereas *Entomoneis alata* produced the maximum amount of chlorophyll a under 25 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, 2.68 pg cell⁻¹.

We also analysed their capacity to produce polyunsaturated aldehydes (PUAs) after artificial cell disruption by sonication and both diatoms were producers: *Nitzschia* produced 2E,4E/Z-Heptadienal and 2E,4E/Z-Decadienal while *Entomoneis alata* produced 2E,4E/Z-Heptadienal and 2E,4E/Z-Octadienal after cell breakage.

UV RADIATION EFFECTS ON ALGAL COMMUNITY STRUCTURE ALONG A LATITUDINAL GRADIENT

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Investigations on photosynthetic characteristics under various light-temperature conditions have revealed that the decrease of photosynthetic capacity from medium to high latitudes corresponds to the decrease of solar irradiance from equator to polar regions. Photosynthetic efficiency appeared to be higher in algae growing in circumpolar areas, by better utilization of solar radiation in low-light environments. E.g. it seems many microalgae are capable of occupying geographic zones that are characterized by specific light-temperature conditions, comparable to what has been observed for numerous macroalgae, and in contradiction to the protist ubiquity hypothesis. Representatives of *Cosmarium* have developed protective strategies to endure extreme irradiation and temperature conditions at various latitudes and altitudes, as well as rapid fluctuations of abiotic factors. Anthropogenic ozone depletion causes an increase of UV-B on the earth surface, a threat to phototrophs not adapted to higher UV-B irradiances. Investigations with algae from tropical and subtropical zones, where UV is naturally higher, are compared with results of polar or species from moderate latitudes growing under lower UV. Photoinhibition was induced using a sun simulator to mimic natural underwater radiation spectrum or by exposure to higher sun radiation close to the water surface. Photosynthetic activity was determined by PAM fluorescence. A comparison of different species and locations of growth showed that the extent to which UV causes an additional decrease of photosynthetic performance during high light stress varies according to the depth of growth and UV transparency of the water. A new finding was that some tropical species were even more strongly inhibited when UV-B was filtered out of the simulated and natural sun spectrum, indicating a supporting effect of the short UV for recovery or against photoinhibition. Thus, UV-B does not solely cause negative effects on photosynthesis i.e. increases photoinhibition, but it may even support recovery processes in aquatic phototrophs adapted to a high UV-radiation environment. The latter is in contrast to earlier

studies, in which UV-B radiation was considered causing only harmful effects on photosynthesis.

MACROALGAE AT PULLEY RIDGE: THE DEEPEST MESOPHOTIC CORAL REEF ON THE U.S. CONTINENTAL SHELF

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Macroalgae are important, but poorly known, components of mesophotic ecosystems (MEs), which are characterized by light-dependent communities typically at depths ranging from 30–40 m and extending to over 150 m in tropical and subtropical waters where water clarity is high. Pulley Ridge, located in the Gulf of Mexico, about 250 km west of the southwest Florida coast, is the deepest mesophotic coral reef on the continental United States. This system, at depths of 60–80 m, is dominated by macroalgae. As part of a broader study of the benthic community of Pulley Ridge, the macroalgal community was characterized by video surveys with remotely operated vehicles during four expeditions from 2012–2015. Mean macroalgal cover was about 46% of the bottom area, far surpassing that of all other biota. The dominant macroalgae were crustose corallines, peyssonnelioids, and *Anadyomene menziesii*, a unique chlorophyte that grows in dense meadows and a species not currently reported from anywhere other than Pulley Ridge. In addition to their important role as primary producers, mesophotic macroalgae are no doubt important to structuring MEs and merit more study to better elucidate and quantify their multiple roles at these depths.

TRANSCRIPTOME ANALYSIS OF COMPATIBLE AND INCOMPATIBLE INTERACTIONS OF ECTOCARPUS WITH EURYCHASMA DICKSONII USING RNA-SEQUENCING

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The widely distributed filamentous macroalga *Ectocarpus siliculosus* serves as a model organism for genetic and genomic approaches in brown algae. *Eurychasma dicksonii*, is one of the most common pathogenic oomycete of brown macroalgae, and infects more than 40% of the tested algal species. Importantly, some host species are resistant (R) to the infection, whereas some are susceptible (S). The understanding of the algal defense mechanisms is unclear. To identify transcriptional mechanisms underpinning the resistance, we analyzed RNAseq data of three different species of *Ectocarpus*: i. *Ectocarpus siliculosus* (genome strain (S)) ii. *Ectocarpus* sp. (R) iii. *Ectocarpus fasciculatus* (R) treated with two strains of *Eurychasma dicksonii*. The sequence reads were aligned to de novo transcriptome assemblies, analyzed to measure gene expression levels in order to identify genes that are differentially expressed between the treatments. The orthologous genes in three different *Ectocarpus* species were identified through PicoPLAZA: 11,094, 1,504 and 696 of the *Ectocarpus* genes were shared across all three species, across *E. fasciculatus* and *E. siliculosus* and across *E. sp* and *E. siliculosus* respectively. We also compared the expression levels of these orthologous genes across the *Ectocarpus* transcriptomes. Using the available high quality genome annotation of *E. siliculosus*, we were able to functionally annotate more than 70% of the *E. sp* and *E. fasciculatus* transcriptomes. Using this approach, we identified classical defense response and detoxification genes i.e. vanadium bromoperoxidase (vBPO), manganese superoxide dismutase (MnSOD), glutathione-S-transferases (GSTs) were induced during resistant interactions. Most interestingly, we also found that the *Ectocarpus* viral-1 genes (EsV-1) were 1-to-2 fold induced/repressed during the compatible/incompatible interactions. Our RNA seq results will

be experimentally tested using the Fluorescent in situ hybridization (FISH) technique for our top 5 candidate gene/gene families. The results will help to identify key resistant/susceptible genes and contribute to a better understanding of the mechanisms of interaction between *Ectocarpus* and *Eurychasma*.

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ENGINEERING A HEAT-TOLERANT AND ECTOIN-PRODUCING MICROALGA IN ONE STRIKE

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Microalgae, including cyanobacteria, have recognised potential for renewable bioproduct and fine chemical production. They can be easily grown in simple outdoor systems with the supply of nutrient-rich wastewaters, with or without additional CO₂ enrichment, in the presence of sunlight. Nitrogen-fixing cyanobacteria can even be grown without the addition of nitrogen fertilisers, as they can convert atmospheric nitrogen to organic nitrogen. So why is commercial production of microalgae still only a reality for high-value commodities, such as the pigments β -carotene and astaxanthin or the heterotrophic production of the long chain polyunsaturated fatty acid docosahexaenoic acid (DHA –for aquaculture, animal feed and infant formulae enrichments)? For outdoor microalgae production in the tropics, high temperatures and variable salinities are the two main limitations to their cost-effective commercial production, as these hurdles are extremely difficult to overcome. Ectoin is a valuable molecule produced by extremophile microbes in response to variable salinities and high temperature stress. As an effective osmolyte, ectoin functions in protecting proteins and other essential biological molecules from dehydration and heat denaturation. Similarly, it protects nucleic acids from UV damage. Thus, ectoin is being increasingly incorporated into skin care products. Our synthetic biology study aimed at engineering de novo a biosynthetic pathway for ectoin production into the cyanobacterium *Synechococcus elongatus* PCC 7942 to examine its effect on the cyanobacterium's temperature and/or salinity tolerance. Our data confirmed increased temperature tolerance to 45°C, while effects on salinity tolerance were moderate in our engineered strain. Liquid

chromatography analysis demonstrated that ectoin was secreted into the medium along with very little production of by-products, making it an easy to harvest and safe product for the cosmetic industry. In addition, the produced biomass could be used for bio-ethanol production or for bio-energy generation via anaerobic digestion due to the high starch content of this cyanobacterial strain.

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LINKING PHYSIOLOGICAL AND TRANSCRIPTOMIC ANALYSES IN DESMARESTIA ANCEPS UNDER FUTURE ABIOTIC CONDITIONS

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Geographical and vertical distribution patterns of macroalgae are constrained by abiotic factors such as light, and including UVR and temperature. Hence, future global environmental changes could have a significant impact on geographic and vertical distribution patterns, as well as primary productivity. Ocean acidification and especially global warming are having a severe effect on polar regions. Brown algae of the order Desmarestiales inhabit rocky shores along the Antarctic Peninsula, forming huge underwater forests which provide habitat and nurseries for various marine organisms. Whereas the basic physiological and ecophysiological characteristics of the endemic macroalga *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. 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 containing 48,265 contigs. Subsequently, physiological and transcriptomic responses of *Desmarestia anceps* to a combination of different levels of temperature (2 and 7°C), dissolved CO₂ (380 and 1,000 ppm), and irradiance (65 and 145 μmol photons m⁻² s⁻¹) have been investigated. Growth and photosynthetic rates increased under high CO₂ conditions, and strongly decreased at 2°C in combination with high irradiance

compared to the rest of the treatments. The highest number of differentially expressed genes was found in response to the 2°C high irradiance ambient CO₂ exposure (5,440), followed by 2°C high irradiance high CO₂ exposure (4,555). High irradiance at 2°C caused a strong repression of genes encoding photosynthetic and ribosomal components. CO₂ enrichment caused only few transcriptomic changes. These results indicate that increased temperature and CO₂ might allow *Desmarestia anceps* to maintain its productivity while tolerating higher irradiances than at present conditions.

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VITAMINS AS DRIVERS OF EVOLUTION OF ALGAL-BACTERIAL MUTUALISMS

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Vitamins are organic micronutrients that are required by organisms because they are the precursors to enzyme cofactors. Many eukaryotic algae, despite their photosynthetic lifestyle, require an exogenous supply of certain vitamins to allow growth, so in that respect they are like animals. More than half of microalgal species surveyed require cobalamin (vitamin B₁₂), over 20% require thiamine (vitamin B₁), and ~5% require biotin (vitamin B₇). There is no phylogenetic relationship between those that require the vitamin and those that do not, suggesting that this has evolved multiple times throughout the algal lineages. Levels of these compounds free in solution in the aquatic environment are frequently very low or undetectable, and there is evidence that at least in some cases, algae obtain the vitamins they need from bacteria in the environment; this is particularly relevant for B₁₂, since this is not made by eukaryotes. Moreover, stable co-cultures of algae and bacteria have been established in the laboratory, where algae receive the vitamins they need directly from bacteria in exchange for some form of fixed carbon. The question arises therefore can vitamin exchange be the means to initiate mutualism? Using genome sequence information we have established the underlying genetic basis for cobalamin auxotrophy in algae, and tested the hypothesis with an experimental evolution approach that resulted in a mutant strain of the B₁₂-independent alga *Chlamydomonas reinhardtii*, which now needs the vitamin; it now forms a stable mutualism with a B₁₂-producing bacterium. At the same time we have screened the genome sequences of over 8,000 bacteria to identify those that might be predisposed to forming mutualisms with algae.

INSIGHTS INTO VITAMIN METABOLISM FROM *PORPHYRA* AND CONSEQUENCES FOR BIOTIC INTERACTIONS

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Porphyra and related genera are commercially important human foods because of their high nutritional value, including being excellent sources of several vitamins. These organic micronutrients are essential components of the human diet, playing vital roles as cofactors of enzymes of central metabolism. To understand better factors contributing to the rich vitamin content of this important edible seaweed, we examined genes of vitamin metabolism in the genomes of *Porphyra umbilicalis* alongside four other red algae: *Chondrus crispus*, *Galdieria sulphuraria*, *Cyanidioschyzon merolae*, and *Porphyra yezoensis*. Surveys for genes involved in vitamin B12 (cobalamin), B1 (thiamine), B7 (biotin) metabolism, alongside those for vitamin E, have provided important insight into these diverse red algae. We obtained evidence for genes of biotin, thiamine and vitamin E biosynthesis, though sequence analysis suggested in some instances these were likely to be pseudogenes. None of the species encode the genes necessary to synthesize cobalamin de novo, as expected since no eukaryote is known to make this vitamin. However, *Porphyra* is a rich source of vitamin B12, indicating that it takes it up from the environment, most likely from its abundant bacterial epiphytes. Vitamin B12 is a required cofactor for B12-dependent methionine synthase (METH), and *Porphyra* encodes both METH and METE, the latter a B12-independent isoform of methionine synthase. This suggests *Porphyra*, does not have an absolute requirement for B12, but may use it if it is available. Notably, *Porphyra* also encodes CobS and CobT, which suggests it can remodel pseudocobalamin, a form of B12 made by cyanobacteria, but which is not bioavailable to algae, nor to humans. Together this work raises interesting questions regarding the selective pressures shaping vitamin pathway evolution in the red lineage of algae, and across photosynthetic eukaryotes more broadly.

MACROALGAL RESPONSE TO A WARMER OCEAN WITH HIGHER CO₂ CONCENTRATION: A SHIFT TOWARDS TROPICAL ECOSYSTEMS

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Primary production and dark respiration rates were studied for six seaweed species (*Cystoseira abies-marina*, *Lobophora variegata*, *Pterocladia capillacea*, *Canistrocarpus cervicornis*, *Padina pavonica* and *Corallina caespitosa*) of rocky bottom areas to estimate the combined effects of pH and temperature variation. Macroalgal samples were cultured at temperature and pH combinations ranging from current levels to those predicted for the next century in a climate change scenario (19°C, 21°C, 23°C, 25°C and 8.1 pH, 7.7 pH, 7.4 pH). Decreased pH had a positive effect on short-term production of the studied species. Raised temperatures had a more varied effect on short term primary production, depending on species. Thermophilic algae with tropical affinity increased their production at higher temperatures, while more temperate species were more productive at lower or present temperature conditions. Temperature also affected algal respiration rates, which were higher at low temperature levels. The results suggest that biomass and productivity of the more tropical species in coastal ecosystems would be enhanced by future ocean conditions.

AIR-SEA CO₂ FLUXES IN MACROALGAL DOMINATED COMMUNITIES

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The ocean helps mitigate climate change due to its large capacity to store CO₂, enabled by the interplay of physical, chemical and biological processes. Ocean CO₂ absorption results in the sequestration of 25% of the total emissions from human activity. The contributions of various vegetated systems to this percentage have been intensively studied. However, the sink capacity of benthic macroalgae and its carbon dynamics are largely unknown. In this sense, the aim of this study was to gain a better understanding of the biogeochemical cycle of carbon in macroalgal dominated ecosystems. Community metabolism was investigated using a minimum of two benthic chambers on four rocky reefs at two different marine protected areas off El Hierro Island and La Graciosa islet (Canary Islands, Spain). The chambers consist of plastic domes enclosing a set of O₂, CO₂, pH and PAR autonomous sensors. Alkalinity was also measured at different times during the 24 h incubation time by extracting seawater from the chambers with syringes. The dynamics of community gross primary production (Pg), respiration (R) and community net calcification (G) were calculated. Then, the daily air-sea CO₂ flux (FCO₂) was calculated using the formula: $FCO_2 = -Pg + R + YG$, Y being the ratio of CO₂ released to CaCO₃ precipitated. The daily FCO₂ obtained at contrasting macroalgae systems was significantly different between studied reefs. La Graciosa islet reefs released CO₂ (1.52/3.53 mmol C × m⁻² × day⁻¹) to the atmosphere while El Hierro island reefs absorbed CO₂ (-45.40/-61.22 mmol C × m⁻² × day⁻¹). This contrasting ability to absorb CO₂ was mainly due to the contrasting dominant macroalgae communities found at each studied area. We believe that the data obtained in this study, as well as the methodology used, is of interest to community ecologist and environmental managers.

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IMPORTANCE OF THE MORPHOLOGICAL VARIABILITY ON PRODUCTIVITY IN MACROCYSTIS PYRIFERA ALONG THE CHILEAN COASTLINE

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One of the most predominant characteristics of the giant kelp forest is its morphological variability, which can explain the wide latitudinal distribution of the species, conquering different environments. This is a descriptive study that allowed us to characterize the morphology of *Macrocystis pyrifera* along the coastline of Chile and relate it to their photosynthetic potential by calculating the photosynthetic area. Five locations were sampled covering the coastline: Algarrobo (33°21'S), Bahía Mansa (40°34'S); Carelmapu (41°44'S); Chiloé (42°32'S); and Punta Arenas (53°35'S). The data collected in each location included density (individuals m⁻²), number of stipes, blade number, length (m) and biomass (g) of 150 adult sporophytes (10 plants × 3 sites × 5 locations) in total. These raw data were used to calculate the Canopy/Subcanopy ratio (C:SC) to establish whether the plant allocates more energy to one or the other. Also, the photosynthetic area was estimated for each location by integrating blade number and blade area for canopy and subcanopy respectively. We found significant differences for all the parameters when comparing the C:SC ratio, with clear latitudinal patterns from N to S for the biomass (H: 15.58; p = 0.004) and length (H: 35.59; p = <0.001). Although the density of plants is higher in the northern location, individuals in the Austral location have a higher overall length, biomass and blade number which is reflected in a higher C:SC ratio and in the photosynthetic area (H: 49.13; p = <0.001). Our results showed that morphology variations can allow for a better use of light by increasing the biomass and photosynthetic area, therefore increasing plant productivity.

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DIVERSITY OF ALGIVOROUS PROTOPLAST FEEDERS AND FIRST INSIGHTS INTO THE MOLECULAR MECHANISMS UNDERLYING THE PERFORATION OF ALGAL CELL WALLS

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Two distantly related groups of amoeboid protists, namely the vampyrellid amoebae (Vampyrellida, Rhizaria) and the viridiraptorid amoeboflagellates (Viridiraptoridae, Rhizaria), display the ability to perforate the cell walls of green algae and to feed on protoplast material. I will provide an overview about known and novel rhizarian protoplast feeders as well as the cell wall characteristics of their algal prey. The viridiraptorid amoeboflagellates, which consume zyg-nematophycean green algae and grow to high cell densities under laboratory conditions, are excellent model organisms to study the molecular mechanisms underlying the perforation of prey cell walls. We used comparative transcriptomics on synchronised, bacteria-free cultures of *Orciraptor agilis* and identified carbohydrate-active enzymes (CAZymes) that are differentially expressed upon contact with the prey alga (*Mougeotia* sp.). Whereas pectate lyase and endo- β -1,4-mannanase (GH5_10) may be involved in the degradation of gel-like cell wall components, a highly up-regulated, plasma membrane-anchored cellulase (GH5_5) with endo- β -1,4-glucanase activity seems to be the key player in the degradation of the tough cellulosic cell wall layer of zyg-nematophycean prey. Future plans to study these enzymes in detail (e.g. substrate specificity) and to localise them in attacking cells of *Orciraptor* will be presented.

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YOU CAN'T GET ME – DEVELOPING HAEMATOCOCCUS STRAINS RESISTANT TO PARAPHYSODERMA INFECTION

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Industrial cultivation of the unicellular green alga *Haematococcus pluvialis* for the natural production of the high value compound astaxanthin is currently affected by the parasite *Paraphysoderma sedebokerense*. *Paraphysoderma* infection causes economic losses, as it reduces the quality of algal biomass and can eventually cause culture crashes. Contaminant-free cultivation is difficult to achieve in large

scale and so far, a publicly available treatment for *Paraphysoderma* infection is lacking.

Paraphysoderma disseminates by motile spores that can encyst on the host surface. Cysts develop into sporangia feeding on the algal protoplast and release a new generation of spores upon maturation, so spreading the disease. In large scale, *Haematococcus* is grown in its green phase which is characterised by a heteromorphic life cycle in which cells transition between haematocysts, palmelloids and macrozooids. The motile macrozooid stage is of particular interest, as it is resistant to *Paraphysoderma* infection. While its abundance during the green phase greatly depends on the *Haematococcus* strain, culture- and environmental conditions, this motile phenotype is normally lacking in the stressful culture conditions that are necessary for astaxanthin production.

In this study, we present the results of a long-term selection experiment in which a highly sensitive *H. pluvialis* strain gained resistance to *Paraphysoderma* infection over several generations of infection by developing a culture phenotype dominated by flagellated cells, not only in its green phase, but also throughout the astaxanthin-accumulating red phase. This acquired resistance thus allows cultivation of *Haematococcus* throughout its whole cycle in presence of *Paraphysoderma*. Monoclonal isolates obtained from the *Haematococcus/Paraphysoderma* co-culture remained flagellated for several months, indicating that the phenotype is stable in absence of the parasite.

This study provides not only a model to study *Paraphysoderma* resistance, it also opens up the possibility to develop *Paraphysoderma* resistant *Haematococcus* strains for cultivation on an industrial scale.

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THE BENEFITS OF DNA-CONTAINING SPECIMENS: CHROOMONAS NORDSTEDTII

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The genus *Chroomonas* (Cryptophyceae) proved to be paraphyletic in previous studies. A revision requires the identification of the type species, though, which is almost impossible in a group of protists with only scarce or non-synapomorphic characters. In *Chroomonas*, Hansgirg fortunately preserved his specimen in a way allowing for DNA extraction and identification of the type species, the real *Chroomonas nordstedtii*.

LATITUDINAL TRENDS IN STABLE ISOTOPE SIGNATURES OF NORTHEAST ATLANTIC RHODOLITHS

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Rhodoliths are free-living calcifying red algae that form extensive beds in shallow marine benthic environments (<250 m) that provide important habitats and nurseries for marine organisms and contribute to carbonate sediment accumulation. There is growing concern that these organisms are sensitive to global climate change, which will have important consequences for coastal productivity and stability. The goal of this study was to determine the plasticity of dissolved inorganic carbon (DIC) uptake mechanisms of rhodoliths along a latitudinal gradient in the Northeast (NE) Atlantic using natural stable isotope signatures. The $\delta^{13}\text{C}$ signature of macroalgae can be used to provide an indication of the preferred inorganic carbon source (CO_2 vs. HCO_3^-). Here we present the total and organic $\delta^{13}\text{C}$ signatures of NE Atlantic rhodoliths with respect to changing temperature and seawater carbonate chemistry along the latitudinal gradient from the Canary Islands to Spitsbergen. We observed a decreasing trend in skeletal $\delta^{13}\text{C}$ signatures with increasing latitude and temperature, while organic $\delta^{13}\text{C}$ signatures were only significantly correlated to DIC. These data suggest that high latitude rhodoliths rely solely on CO_2 as an inorganic carbon source, while low latitudes rhodoliths may be able to utilize HCO_3^- . However, depth also has a significant effect on both skeletal and organic $\delta^{13}\text{C}$ signatures, suggesting that both local and latitudinal trends influence the plasticity of rhodolith inorganic carbon acquisition and assimilation. Our results show that many species, particularly those at lower latitudes, have carbon concentrating mechanisms that facilitate HCO_3^- use for photosynthesis. This is an important adaptation for marine macroalgae, because HCO_3^- is available at higher concentrations than CO_2 in seawater, and this becomes even more extreme with increasing temperature.

RHODOLITHS MAINTAIN ELEVATED SURFACE PH AND CARBONATE ION CONCENTRATIONS

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Red coralline algae are projected to be sensitive to ocean acidification, particularly in polar oceans where the carbonate buffering capacity is weak. These algae play key roles as ecosystem engineers in global oceans; thus their potential sensitivity to global climate change is worrisome, and understanding their calcification mechanism is necessary for making reliable predictions and subsequent policy decisions. Therefore we investigated the localized carbonate chemistry at the surface of Arctic rhodoliths and north Atlantic maërl using microsensors. We report for the first time surface carbonate ion concentration and pH measurements ($[\text{CO}_3^{2-}]$) in the microenvironment of rhodoliths. We show that surface pH and $[\text{CO}_3^{2-}]$ in rhodoliths are higher than the bulk seawater in the light, and Arctic rhodoliths maintain this condition even after hours of darkness. We also report the localized carbonate chemistry at the surface of north Atlantic maërl exposed to ocean acidification (1,200 ppm CO_2) for one month. Our results suggest that rhodoliths have strong biological control over their surface chemistry, even under ocean acidification conditions. Our results provide further insight into how coralline algae regulate the seawater chemistry in their microenvironment and how this might change under future ocean acidification.

ARCTIC AND ALPINE ZYGNEMA SP. (ZYGNEMATOPHYCEAE) ADAPT TO DESICCATION, UV RADIATION AND FREEZING: INVESTIGATION OF ULTRASTRUCTURAL AND METABOLOMIC CHANGES

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Conjugating green algae (Zygnematophyceae) are considered to be closest algal relatives to land plants (Embryophyta) and therefore their common ancestor is supposed to have had adaptations that enabled colonization of land. Today, several members of this group still typically grow in harsh aeroterrestrial environments. In Polar or high Alpine regions, some filamentous Zygnematophyceae often dominate and typically produce relatively large amounts of biomass. They however have to withstand desiccation, freezing and UV radiation in their natural environment.

Therefore, these organisms have developed good resistance mechanisms, and form modified vegetative cells so-called pre-akinetes. These are permanent stages, rich in storage compound and lipids that develop during the summer season, leading to winter survival. Fatty acid composition shows a significant change upon pre-akinetes formation. Metabolomics of young vegetative cells and pre-akinetes show a clear shift of metabolites during this transition, e.g. osmotically active substances increase. When desiccation effects were investigated by transcriptomics, an up-regulation of protection mechanisms like ROS scavenging, DNA-repair, chaperons and aquaporins was found. In sun-simulation experiments with increased UV-AB radiation, a high tolerance was found in pre-akinetes. On the metabolite level, the different strains (alpine *Zygnema* S, arctic *Zygnema* B and Antarctic *Zygnema* C) as well as vegetative vs. pre-akinetes stage were clearly distinguishable. For UV-tolerance, enhanced levels of phenolic compounds are beneficial. Freezing experiments illustrated that young vegetative cells were able to tolerate only slight freezing temperatures, whereas pre-akinetes could tolerate freezing down to -30 or -40°C . In addition to pre-akinetes, zygospores that result from conjugation can be formed, which is a rare event in arctic samples, and was only observed in the closely related genus *Zygnemopsis* sp. With these adaptation mechanisms, *Zygnema* sp. are well prepared to live in terrestrial habitats.

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DNA-BASED SPECIES DELIMITATION AND CROSSING EXPERIMENTS ON THE SCYTOSIPHON LOMENTARIA SPECIES COMPLEX (ECTOCARPALES, PHAEOPHYCEAE) FROM JAPAN

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Japanese populations of *Scytosiphon lomentaria* have been suggested to consist of multiple sibling species. In this study, we re-examined the Japanese *S. lomentaria* species complex, using three quantitative DNA-based species delimitation analyses (GMYC, PTP and ABGD) on mitochondrial (*cox1*) and nuclear (second intron of centrin gene) DNA sequence data sets. The delimitation analyses suggested the existence of five putative species (species I–V) in Japanese *S. lomentaria*, and these species were often found in the same locality. To test the DNA-based species boundaries and to reveal the reproductive barrier, crossing experiments were conducted among the five putative species using culture isolates. As a result, gametic incompatibility, which prevented hybrid zygote formation, was observed among the five putative species, except for the crosses between species II male and species III female, in which hybrid zygotes were successfully formed (asymmetric hybridization). These hybrid zygotes grew to sporophytes and matured with unilocular sporangia in culture. However, the survival rate (49.3%) of spores (meiospores) from the hybrid sporophytes was significantly lower than that (91.5%) of spores from sporophytes derived from intra-species crosses. The spores from the hybrid sporophytes often stopped growing at the early stage (one- to three-celled germlings) of development and died. The biological boundaries suggested by our crossing experiments were consistent with the well-supported DNA-based species boundaries. Gametic incompatibility is probably the main reproductive isolation mechanism among the five species in sympatric localities. In addition, disorder of meiosis in the hybrid sporophytes could function as a reproductive barrier.

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FEMALE-DOMINANT ASEXUAL POPULATIONS OF THE BROWN ALGA SCYTOSIPHON LOMENTARIA IN JAPAN

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The dioecious and isogamous brown alga *Scytosiphon lomentaria* (Scytosiphonaceae, Ectocarpales) is widely distributed in temperate and cold coasts around the world. *S. lomentaria* found in Japan consists of five cryptic species: species I–V. In species I, both sexual and asexual populations have been reported. In this study, the sex of gametophytes from asexual populations was checked using sex markers and by crossing experiments with female and male gametophytes from sexual populations. All gametophytes from the asexual populations were revealed to be female. In culture, gametes released by gametophytes from the asexual populations were compared with gametes released by gametophytes from the sexual populations. Gametes from the asexual populations had a significantly larger cell size and rapid parthenogenetic development. Although gametophytes of the asexual populations were suggested to be “female,” the cultures where their gametes were released had no detectable smell of their sexual pheromones. Furthermore, sporophytic discoid thalli, which are considered to be developed by parthenogenesis, were found at an area where an asexual population exists. These results suggest that the asexual populations consist of “females” that have a high reproductive ability in parthenogenesis, and that, an alternation of generations between macrothallic gametophytes and microthallic discoid sporophytes occurs through parthenogenesis of female gametes without sexual reproduction in the asexual population.

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A LATE PLEISTOCENE MARINE GLACIAL REFUGIUM IN THE SOUTHWEST OF HAINAN ISLAND, CHINA: PHYLOGEOGRAPHIC INSIGHTS FROM THE BROWN ALGA SARGASSUM POLYCYSTUM

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Hainan Island, southern China, is characterized by rich diversity and endemism of marine organisms. However,

the underpinning mechanisms and processes contributing to speciation and diversification are poorly investigated in terms of historical biogeography. Here, we chose the brown alga *Sargassum polycystum* as a model to explore the biogeographical history of Hainan Island and identify putative glacial refugia in the late Plesitocene ice ages. We analyzed mitochondrial *cox1* and *cox3* and nuclear internal transcribed spacer-2 (ITS2) of 310, 325 and 313 individuals of *S. polycystum*, respectively. Phylogenetic trees, haplotype/ribotype networks, analysis of molecular variance (AMOVA), neutrality tests, current ($\theta\pi$) and historical (θw) genetic diversities and extended Bayesian skyline plots (EBSP) showed that the populations from the southwest of Hainan Island harboured much higher genetic diversity and unique endemism in comparison with other populations in the distribution range. *S. polycystum* experienced relatively long-term stable population size followed by a continued period of demographic expansion in the late Pleistocene.

Our phylogeographical evidence revealed the existence of a previously unidentified marine refugium in the southwest of Hainan Island, China (the Central Depression of the Yinggehai Basin), along with a possible secondary refugium around the Bali Island, Indonesia. These biogeographical scenarios empirically provide important insights regarding speciation, adaptation and evolution of marine organisms in Southeast Asia and the conservation of unique biodiversity under climate change.

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SUSTAINABILITY AND LESSONS LEARNED FROM EUCHEUMATOID FARMING

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Since 2009, *Kappaphycus* and *Eucheuma*, known collectively as the eucheumatoid algae, became the largest, cultivated seaweed biomass on a global scale. These carrageenophytes overtook production of the brown seaweeds *Laminaria* and *Undaria* according to published FAO data. The Southeast Asian region, in particular Indonesia, the Philippines, Malaysia and in Africa, Tanzania have produced commercial quantities of eucheumatoids from 1971 to date.

From the successful stories of sustainable, red seaweed, agronomy, there are multiple best practices which can be

transferred as recommendations for future socio-economic development and prosperity in rural, coastal economies. For example, the use of repeated, vegetative propagation of *K. alvarezii* with its inherent low genetic variation resulted in widespread susceptibility to disease and epiphyte infestations; the introduction of non-indigenous eucheumatoids into new farming areas needs to be carefully considered; and even after more than 40 years, farming is still dependent on rudimentary, repetitive, intensive labour (e.g. individual cuttings tied on to lines); there have been developments in newly introduced tubular net bags in Brazil and India. R&D has generally stagnated in the carrageenan industry focusing on commoditized, processed food applications, however, recently adopted multi-stream zero effluent (MUZE) processing is slowly gaining acceptance and receiving commercial investment in India and Indonesia.

A shift from simple cultivation to extensive agronomy for eucheumatoids is imperative in order to sustain strategies such as : 1) enhancement of human capacity; 2) diversified livelihoods; 3) sound, ecosystem- based management; 4) providing resilience to climate change; and 5) secured sustainability. All of these aspects will be presented in this paper.

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TIPPING THE BALANCE: IRON AND PHOSPHORUS CO-LIMITATION OF CYANOBACTERIAL N₂ FIXATION IN THE PRESENT AND FUTURE OCEAN

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The conventional algal nutrient limitation paradigm is based on Liebig's Law of the Minimum, whereby the single nutrient in shortest supply limits marine phytoplankton production. However, we now know that co-limitation is common in marine primary producers, with two or more resources simultaneously limiting growth and biomass. Co-limitation can be easily demonstrated with basic growth rate measurements, but we understand very little about the biochemical cross-talk between multiple nutrient acquisition, utilization and storage pathways. I will present our work that uses approaches ranging from physiology to transcriptomics and proteomics to understand the biochemistry and biogeochemistry of co-limitation in the marine nitrogen-fixing cyanobacterium *Trichodesmium*. We discovered a unique iron (Fe) and phosphorus (P) co-limitation phenotype that provides a surprisingly potent fitness advantage over limitation by either iron or phosphorus alone, suggesting that

Trichodesmium is much better adapted to growing under 'balanced limitation' by both nutrients at once than it is to Liebig-type single nutrient limitation. I will then discuss the interactions of balanced Fe/P co-limitation with long-term adaptation to high CO₂, with implications for nutrient limitation dynamics in the future acidified ocean. Nitrogen fixers appear to have been finely tuned by evolution to thrive in the oligotrophic ocean under chronic multiple resource co-limitation, and this adaptation will in turn alter their responses to increasing atmospheric CO₂. Our work suggests a need to fundamentally reassess some of the long-standing nutrient paradigms in phytoplankton biology for both the current and future ocean.

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TRANSCRIPTOME-BASED IDENTIFICATION OF THE DESICCATION RESPONSE GENES IN THE MARINE RED ALGA PYROPIA TENERA (RHODOPHYTA) AND ENHANCEMENT OF ABIOTIC STRESS TOLERANCE BY PTDRG2 IN CHLAMYDOMONAS

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Pyropia tenera (Kjellman) is a marine red alga which grows in the intertidal zone and loses more than 90% of its water during low tides every day. In order to identify the desiccation response gene in *P. tenera*, we generated 1,444,210 transcriptome sequences, using the 454-FLX platform, from the gametophyte under control and desiccation

conditions. De novo assembly of the transcriptome reads generated 13,170 contigs, covering about 12 Mbp. We selected 1,160 differentially expressed genes (DEGs) in response to desiccation stress based on Reads per Kilobase per Million Reads (RPKM) expression values. As shown in green higher plants, DEGs under desiccation are composed of: two groups of genes for gene regulation networks, functional proteins for carbohydrate metabolism, membrane perturbation, compatible solutes and specific proteins similar to higher plants. DEGs that show no significant homology with known sequences in public databases were selected as desiccation response genes (DRGs) in *P. tenera*. PtDRG2 encodes a novel polypeptide of 159 amino acid residues locating to the chloroplast. When PtDRG2 was over-expressed in *Chlamydomonas*, the PtDRG2 conferred mannitol and salt tolerance in transgenic cells. These results suggest that *Pyropia* may possess an additional pathway or novel genes which differ from green plants, although the desiccation tolerance mechanism in red algae is similar to that of higher green plants. These transcriptome sequences will facilitate future studies to understand the common processes and novel mechanisms involved in desiccation stress tolerance in red algae.

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EFFECT OF PUTRESCINE ON METHYLATION IN CHLAMYDOMONAS REINHARDTII UNDER SALT STRESS

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It is suggested that putrescine is necessary for acclimation under salt stress stress. The effect of salt stress on some physiological parameters (growth rate, lipid peroxidation, and Rubisco protein amount), methylation rate and gene expression of cytosine-C5 specific DNA methyltransferase (*dmt1*) gene were investigated in the green alga *Chlamydomonas reinhardtii*. Our data indicated that the addition of exogenous putrescine significantly enhanced Rubisco protein amount and decreased lipid peroxidation level during salt stress. In addition, analyses of the gene expression with Real-time RT-PCR results showed putrescine induced *dmt1* gene expression. In addition, the DNA methylation rate was detected using FTIR analysis. Our results indicated that putrescine could be involved in methylation under salt stress.

¹³C LABELLING FOR METABOLIC FLUX ANALYSIS IN HETEROTROPHIC EUGLENA GRACILIS

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The metabolism and biochemistry of *Euglena*, a class of green protozoan alga, has long been a focus of research. It has been studied as a promising dietary supplement, a host for bioactive compound synthesis, a source of bio-energy, a bioindicator and for treatment of wastewater. However, due to the lack of genome sequencing data, the metabolic capabilities of *Euglena* have not yet been elucidated. In order to explore the metabolic capacity of *Euglena*, we shall combine transcriptome sequencing results with ¹³C metabolic flux quantification based on isotopic labelling (metabolic flux analysis – MFA). This approach will be used to reveal the metabolic phenotype of *Euglena* in terms of principal biochemical pathways operating in vivo.

Heterotrophically grown *Euglena gracilis* was chosen as the model organism for this study, because of the abundant studies of its biochemistry and the availability of the transcriptome, required for metabolic network reconstruction. The *E. gracilis* cells were cultured using glucose as a sole carbon source for isotope labelling experiments and the biomass composition of the cell in this growth condition was analysed. The culture was then fed with 99% [1-¹³C] glucose, 99% [2-¹³C] glucose and a mixture of 20% [1³C-6] glucose and 80% unlabeled glucose and then analysed using GC-MS. The protocols involved in ¹³C MFA in *E. gracilis* were carefully developed and some significant factors in labelling data analysis were validated. These data provide the basis for ¹³C MFA of *E. gracilis*, giving insight into its carbon metabolism.

METAMORPHOSIS IN CYANOBACTERIA: HOW TO CHANGE A GENUS IN SEVERAL DAYS

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Cyanobacteria with true branching are classified to Nostocales. They possess the most complex morphology among cyanobacteria, but it is also one of the least studied groups regarding molecular evolution, taxonomy, and distribution. This remarkable group is characterized by a presence of heterocysts and by three types of true branching (i.e. *Fischerella* and *Hapalosiphon* possess T-type, *Mastigocladus* characterized by Y-type or *Iphinoe* with V-type branching). Recent research has shown that many genera, e.g. *Fischerella*, *Hapalosiphon*, and *Mastigocladus*, have a polyphyletic origin. Moreover, morphological features often overlap among these genera. To elucidate evolutionary trajectories within the true branching cyanobacteria, we isolated two strains of cyanobacteria from a hot water spring in a Java rainforest. The strains exhibited morphological features similar to *Fischerella*. We obtained molecular markers such as 16S rRNA and performed phylogenetic inference. The strains were also subjected to a nitrogen gradient experiment to investigate its effect on morphology. We found that our strains showed morphological features typical either for *Fischerella* or *Hapalosiphon* depending on nitrogen concentration.

THE AUSTRALIAN NATIONAL ALGAE CULTURE COLLECTION – ANACC

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The Australian National Algae Culture Collection, ANACC, maintains over 1,000 strains of microalgae and cyanobacteria sourced from biodiscovery throughout Australia as well as complementary global strains used for comparative taxonomic and chemophenotypic characterizations. ANACC is the national repository for microalgae from Australian waters. Core strategies of ANACC include documenting and digitizing Australia's microalgae biodiversity with an emphasis on species/strains of economic importance, both positive and negative, including bioapplications and harmful algal bloom (HAB) species. Eco-physiological research has proven valuable to understanding environmental issues as well as bioapplications potential. Morphological, multigene genomics and chemotaxonomy (qualitative and quantitative photosynthetic pigment and lipid analyses) are used to characterize ANACC strains. Bioapplications studied include aquaculture, omega-3 oils, biofuels, unusual lipids, pigments, and exopolysaccharides. ANACC biodiscovery is exemplified by two very different microalgae. Firstly, we identified strains of a novel Tasmanian sourced *Nannochloropsis* (Eustigmatophyte) species, *N. australis* and, after examining global genomic biogeography, have proposed a new genus, *Microchloropsis* for the existing species *N. gaditana* and *N. salina*. The second documents novel hydrocarbon chemistry and morphological plasticity within the high oil producing freshwater chlorophyte *Botryococcus braunii* collected throughout Australia. ANACC is the major supplier of live microalgae feeds to the Australian aquaculture industry, supplying microalgal cultures to over 70 countries through its commercial arm, the Australian National Algae Supply Service – ANASS. ANACC works intimately with multidisciplinary research groups in CSIRO in bioapplications research including wastewater strain characterization, growth optimization and gene discovery, and novel harvesting of transformed microalgae from photobioreactors.

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DINOFLAGELLATE PLASTIDS AS MODELS FOR UNDERSTANDING ENDOSYMBIOSIS

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How and when dinoflagellates acquired their plastids has been a matter of ongoing discussions. About half of the described species are non-photosynthetic and traditionally thought to lack plastid organelles. But photosynthesis has a complex distribution in the group and in some species, light harvesting by the archetypal peridinin-pigmented plastid has been substituted by more recent endosymbionts or kleptoplasts. Multiple lines of evidence now suggest that the peridinin plastid was inherited from a common ancestor of dinoflagellates, perkinsids, apicomplexans and chrompodellids and thus photosynthesis in the broader group has shaped biology of aquatic systems at least since the early Paleozoic. Sequence data-based reconstruction suggests that many non-photosynthetic dinoflagellates have retained nucleus-encoded genes of endosymbiotic origin with plastid targeting signatures. Critically, alternative cytosolic pathways for essential cellular metabolites (of host origin) have also been lost, most centrally the biosynthesis of isoprenoid subunits. This makes non-photosynthetic dinoflagellates dependent on plastid-localized metabolism even when photosynthesis is lost. Plastids are predicted to be present in heterotrophs (e.g., *Noctiluca* and *Oxyrrhis*), osmotrophs (e.g., *Cryptothecodinium*) and even species with secondary symbionts (e.g., *Durinskia*) and kleptoplastids (e.g., *Dinophysis*). Plastid metabolism may also be linked to key aspects of dinoflagellate biology, including the biosynthesis of tetrapyrroles, sterols and luciferin-based bioluminescence. Altogether, the evidence supports the prediction that all free-living dinoflagellates have retained remnant plastids. This provides an unparalleled model for understanding dependency on plastids in eukaryotes of algal ancestry as a whole. More recent acquisition of endosymbionts in the group have special significance. Rampant replacement of endosymbionts, such as those in the *Kryptoperidinium-Durinskia* clade, may provide a rare model for

studying early steps in the establishment of endosymbioses, particularly with regards to synchronous symbiont-host division, nuclear ploidy and endosymbiotic gene transfer.

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A LIPID RAFT-MEDIATED ENDOCYTOSIS IS INVOLVED IN HOST CELL ENTRY OF AN OOMYCETE PATHOGEN *OLPIDIOPSIS PYROPIAE*

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Host cell entry is the most crucial infection step in the obligate parasite, *Olpidiopsis pyropiae*, a causative agent of *Olpidiopsis* blight in *Pyropia* farms. Oomycete and fungal effectors with RXLR and RXLR-like motifs can bind phospholipids, specifically phosphatidylinositol-3-phosphate (PtIns-3-P), and mediate cell entry via lipid raft-mediated endocytosis. The entry of *Olpidiopsis pyropiae* cell into the host, *Pyropia yezoensis*, was blocked with the treatment of PtIns-3-kinase inhibitors. PtdIns-3-P binding proteins and inositides that competitively inhibit effector binding to cell membrane also inhibited infection. Fluorescent labeling showed changes of PtdIns-3-P distribution along the cell surface during the infection. Homologues of PtIns-kinase and phosphatidylinositol-binding clathrin assembly protein (PICALM) were isolated from *P. yezoensis* transcriptome, and their expression profiles during the infection process were observed using qPCR. Our results show that the oomycete pathogen, *Olpidiopsis pyropiae*, may use a lipid raft-mediated endocytosis for its host cell entry.

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NUNDUVA, A NEW MARINE GENUS OF RIVULARIACEAE (NOSTOCALES, CYANOBACTERIA) FROM MARINE ROCKY SHORES

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Several populations of a non-tapering, fasciculated, geminately false branched heterocytous cyanobacterium were collected from rocky shores in the Pacific Ocean in Oaxaca, Mexico. The populations were provisionally placed in *Brasilonema* based on morphology, but upon sequencing of both environmental and culture material it was discovered that the populations/cultures belonged to the Rivulariaceae, in a tropical marine subclade of the family containing *Kyrtuthrix huatulcensis*. In culture, the taxon exhibited tapering in isopolar filaments, providing further evidence that it was a member of the Rivulariacean clade. Upon examining a number of isolates from both the Pacific and Atlantic coasts of Mexico, as well as an isolate from Great Britain, we concluded that all belonged to one generic cluster. We intend to describe the species cluster “*Nunduva*”, a sister taxon to *Kyrtuthrix*. “*Nunduva*” presently contains at least four morphologically and phylogenetically distinct species that require description.

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THE IDENTITY OF ENIGMATIC SILICEOUS MICROFOSSILS IN MARINE SEDIMENTS

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Microfossils in marine sediment and outcrop samples, particularly deep-sea drilling materials, are routinely observed with the light microscope, often with the primary aim of providing age dating. These observations may be cursory, limited to the recognition of zone fossils. As such, rare or unusual specimens are frequently overlooked, particularly mineralised scales less than 10 μm in size or elongate spines. An investigation of siliceous microfossils in samples of various ages using the scanning electron microscope revealed the presence of a number of enigmatic or rare specimens. Of the siliceous scales, one appears to be a fossil parmalean alga from the Eocene, while some of those from the Eocene and Miocene belong to haptophytes (assignable to *Hyalolithus*, originally described from modern seas). Yet other siliceous scales and skeletons remain enigmatic (e.g., *Macrora* spp. from the Eocene-Miocene and *Clathropyxidella similis* and *Cannopilus jouseae* from the Eocene and Miocene, respectively) or are clearly of non-algal origin (e.g., the scales of a *Pinaciophora*-like species (filose amoeba) found in the Oligocene and the *Chaetoceros* seta-like spines of fossil *Sticholonche* spp. (Radiolaria) from the Oligocene-Miocene). Some of these groups, until now, did not

have a known fossil record, so our observations may shed light on their evolution.

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SALICYLIC-ACID-MEDIATED DISEASE RESISTANCE IN PYROPIA YEZOENSIS AGAINST OOMYCETE PATHOGENS

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Pyropia sea farms suffer from annual outbreaks of oomycete diseases such as red-rot disease caused by *Pythium porphyrae* and *Olpidiopsis* blight caused by several species of *Olpidiopsis*. Oomycete infection in *Pyropia* occurs via asexual zoospores penetrating into the host cell. Our previous studies showed that external calcium level affects the zoosporangium development as well as zoospore release in oomycete pathogens. We hypothesized that salicylic acid treatment can disturb calcium signaling in the pathogen and enhance host immunity. Inhibition experiments showed that calcium salts effectively inhibit the development of zoosporangium and release of zoospores of *P. porphyrae*. The infection rate decreased to 14.3% compared to the control in 3 days after the application of 0.1 M calcium propionate on infected *Pyropia yezoensis* for 30s. Salicylic acid (SA) was applied to the host thallus to increase the immunity and activate the systemic acquired resistance of *P. yezoensis*. When the *P. yezoensis* blade was incubated in SA for 2 hr prior to incubation with oomycete pathogens, the infection rate dropped to 30% of the control. When SA and calcium propionate were applied together almost no oomycete infection was observed. Homologues of a calcium binding protein kinase were isolated from the transcriptome of *P. yezoensis*, and their expression was observed with SA treatment. Our results suggest that calcium ions and calmodulin may regulate SA-mediated disease resistance in *P. yezoensis*.

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DIATOM FLORA OF BRITAIN AND IRELAND – A NEW ONLINE TOOL FOR DIATOM IDENTIFICATION

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Diatoms are important for freshwater ecological assessments for the Water Framework Directive, but no modern diatom flora covering Britain and Ireland exists. Approximately 2800 species of freshwater diatoms have been recorded from Britain and Ireland, with significant taxonomic revisions taking place over the last 20 years. The British Diatomaceae by William Smith (1853, 1856) represents the only explicitly British diatom flora (marine, brackish, freshwater taxa). More recent publications include an account of British marine diatoms by Hendey (1964), a checklist of recorded diatom species by Hartley (1986), the atlas of diatom illustrations by Sims (1996), a coded taxa checklist by Whitton et al. (1998), and a CD-based key to river diatoms (Environment Agency 2007). The UK ring test exposed recurring problems with diatom identification which reduce the accuracy and precision of ecological assessments, and highlighted the need for a new identification resource. The website 'Diatom Flora of Britain and Ireland' is an online identification tool for researchers with an interest in diatom taxonomy and ecology. It documents taxa found in Britain and Ireland using light and electron microscope images and incorporates recent taxonomic revisions. The website is hosted by Amgueddfa Cymru – National Museum Wales (<http://naturalhistory.museumwales.ac.uk/Diatoms>). The British Phycological Society provided funding for a team of editors to meet for workshops and visit collections to compile data and images for the website. Descriptions and literature references are currently available for almost 200 taxa with an emphasis on those frequently encountered during water quality assessments. The website will be updated continuously. From 2017 onwards ecological information will be added, a glossary section started, and factsheets for species will be available to download. We will also add names and publication details for species similar to the taxa documented on the website, whether found in the British Isles or not.

RECOVERY FROM ACIDIFICATION AND RESTORING OF FISH POPULATIONS IN THE CATCHMENT OF THE RIVER WYE, UNITED KINGDOM

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The Welsh River Wye was once the most productive salmon river in England and Wales, but over the last decades numbers of fish have declined dramatically. In the upper catchment, continued deposition of anthropogenically derived acidifying compounds had significant negative effects on salmonid numbers in areas where conifer plantations are the dominant land use. Despite chemical recovery from chronic acidification, long-term monitoring datasets of water chemistry and diatom assemblages from the upper Wye and the Irfon, one of its main tributaries, showed that episodic acidification continued to threaten the recovery of acid-sensitive biota and the recolonization by salmonid fish. The Wye Powys Habitat Improvement Scheme (PHISH) project and the Irfon Special Area of Conservation project aimed to combat acidification through liming in large-scale B-A-C-I (before-after, control-impact) experiments. In total 50 sites including downstream transects on the River Wye and on the River Irfon, and several tributaries to both rivers were monitored three to four times per year over 10 years. There were large pH variations prior to liming, reflecting acid episodes, with pH values often below 5.5 during high flow in areas with conifers. Diatom assemblages were dominated by acid tolerant diatom species such as *Eunotia exigua* and *E. subarcuatoides*. Two methods of liming were tested, hydrological source liming on the River Wye and sand liming on the River Irfon, with the latter being more effective and leading to a significant increase in pH during high flow. Diatom assemblages changed post-liming: acid sensitive species such as *Achnanthydium minutissimum* and *Fragilaria gracilis* increased while *Eunotia* species declined. This was followed by the establishment of salmon populations in previously depopulated sections of the River Irfon.

AN OVERLOOKED GENUS “OLIFANTIELLA C. RIAUX-GOBIN & COMPÈRE”. EXPANDING BIOGEOGRAPHY WITH NEW SPECIES AND ULTRASTRUCTURE OBSERVATIONS

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The small-sized marine diatom genus *Olifantiella* was originally described from the tropical waters of Réunion Island as *Olifantiella mascarenica* C. Riaux-Gobin & Compère. Later, more species were found from the South Pacific and Indo-Pacific and from Port of Antwerp, Belgium expanding the biogeography. Genus *Olifantiella* have some distinct characters; single row of areola (macroareola), trumpet like structure “buciniportula” in internal valve and broad perforated girdle. It is easily overlooked, according to Giffen after ultrastructure examination of *Navicula* species, some were found to share the same characters. *Navicula infirmitata* was described from South Africa by Giffen and *Navicula supralitoralis* described by Aleem & Hustedt from southern England. These two taxa were also reported from

the Baltic Sea under the genus name “*Navicula*”. These species are proposed for transfer into *Olifantiella* in terms of possessing macroareola and buciniportula. In addition, LM and SEM studies revealed new *Olifantiella* taxa in scrapes from loggerhead sea turtle (*Caretta caretta* Linnaeus, 1758) carapaces sampled from the Turkish coast nesting grounds. Here, we introduce two epizoic species new to science.

More *Olifantiella* species have been recorded with ultrastructure examination and Ion beam fabrication. *O. pseudobiremis* is recorded from Black Sea coasts, whilst *O. mascarenica* is recorded in Aegean Sea and Adriatic Sea for the first time. These results expand the distribution of *Olifantiella* from tropical to temperate waters.

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REDUCTIVE EVOLUTION OF PLASTIDS IN DIATOMS AFTER LOSS OF PHOTOSYNTHESIS

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Diatoms are known as one of the major primary producers in aquatic environments. However, some diatom species have relatively recently lost photosynthesis and thus are colorless. Evolutionary scenarios and their genetic background after loss of photosynthesis in diatoms remained unclear. In this presentation, I introduce diversity and evolution of non-photosynthetic plastids of *Nitzschia* spp. I and my colleagues have isolated and established non-photosynthetic diatom strains comprised of 6 morphologically distinguishable lineages from the mangrove estuaries in Ryukyu Islands, Japan. Large subunit rRNA gene phylogeny of *Nitzschia* and related genera divided non-photosynthetic diatoms into several groups that were not united together. Transmission electron microscopic observation unveiled four membrane-bound non-photosynthetic plastids with reduced thylakoid-like structures. I and my colleagues determined the complete plastid genome sequences of one strain each from four distinct lineages, and the genomes were almost two thirds as small as those in photosynthetic relatives in size. They commonly lack any genes for photosystems I and II, cytochrome b6/f complex, chlorophyll synthesis, carbon fixation, and most genes for other metabolic pathways. Most importantly, however, they all retain genes for ATP synthase complex,

in addition to genes for Fe-S cluster assembly, protease, expression, and protein translocators into the thylakoid lumen. Transcriptome analyses and heterologous localization studies revealed that, even after loss of photosynthesis, the non-photosynthetic diatom plastids function for biosynthesis of amino acids and fatty acids and for glycolysis and the reductive pentose phosphate pathway, the latter two which share enzymes with the Calvin-Benson cycle without carbon fixation steps. The non-photosynthetic plastid serving various metabolites would still help the non-photosynthetic diatoms thrive as heterotrophic osmotrophs.

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MARGINAL DISTRIBUTION AND HIGH HETEROZYGOSITY OF ASEXUAL CALOGLOSSA VIEILLARDII (DELESSERIACEAE, RHODOPHYTA) ALONG THE AUSTRALIAN COASTS

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It is known that many asexual species of terrestrial organisms are caused by interspecific or interpopulational crossings and that such heterozygous asexuals are frequently seen in more marginal habitats than their sexual relatives. Although asexual species have been reported in various macroalgal taxa, detailed information about distribution, heterozygosity and origin are limited. Because many asexual tetrasporophyte strains of *Caloglossa vieillardii* had been isolated from South Australia, far from their core tropical habitats, we examined the distribution range of asexual *C. vieillardii*, and genotyped them and other western Pacific strains using a single-copy actin gene. The asexuals were distributed at the western edge of sexual populations, from Adelaide to Arno Bay. While only one asexual strain was a homozygous genotype and sympatrically distributed with the sexual relatives, all other asexual strains were heterozygous and distributed far from the sexual populations. It was assumed that the most abundant genotype in the asexual strains, heterozygous Type 3/4, originated from the outbreeding between Type 3 and Type 4 sexual plants, both of which were found from the Australian coasts. In the crossing experiments using some sexual strains, certain combinations between Type 3 female and Type 4 male produced tetrasporophytes with spermatangial sori, which

recycled successive tetrasporophytes. The culture experiments showed that both sexual and asexual strains successfully produced tetraspores at 13°C but no sexual strains released carpospores below 14°C. Because seawater temperature falls to below 14°C around the asexual populations of *C. vieillardii*, sexual thalli may not be able to complete their life cycle there.

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CRISPR/CAS9-INDUCED KNOCKOUT IN UNICELLULAR ZYGNEMATOPHYCEAN ALGA, CLOSTERIUM PERACEROSUM-STRIGOSUM-LITTORALE COMPLEX

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We have focused on the *Closterium peracerosum-strigosum-littorale* complex (*C. psl.* complex), belonging to the order Zygnematales. It is the most widely studied unicellular charophycean alga in terms of the maintenance of strains and sexual reproduction. A technique for genetic stable transformation has been developed and its genome project is now underway.

We have found 88 pheromone-inducible, conjugation-related and/or sex-specific genes through cDNA microarray analyses. Among them, we have focused on the *CpRLP1* gene encoding a leucine-rich repeat (LRR) receptor-like protein. The function of *CpRLP1* was characterized using knockdown mt- transformants of *CpRLP1* by the expression of antisense RNA. However, the inhibitions of the protein expression were incomplete and the sexual phenotypes were varied among transformants.

To evaluate the function of the unknown gene during sexual reproduction, we developed the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated proteins 9 (Cas9) system (CRISPR/Cas9 system) for the *C. psl.* complex. We constructed a vector for the system using an endogenous CpU6 gene promoter and a codon-optimized Cas9 gene. Using this vector, we succeeded in establishment of the frame shift knockout strains for three independent genes (*CpRLP1*, *CpRLK2*, and *CpMinus1*). In the case of *CpRLP1*-knockout mt- strains, the conjugating

reaction was severely reduced and a hyper-sensitive reaction to the PR-IP was observed. In contrast to the *CpRLP1*, the *CpRLK2*- knockout mt- strains, did not show the mating reaction and did not respond to the PR-IP. The *CpMinus1* is a mt- genome-specific gene encoding a transcription factor. The knockout mt- transformants showed mt+- like behavior. These phenotypes were consistent among respective transformant lines. This is not just the first successful gene knockout in Zygomatales, but is the first report in charophyceans.

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TRANSFER FOUR SPECIES
OF HARALDIOPHYLLUM
(DELESSERIACEAE, RHODOPHYTA)
TO NEOHARALDIOPHYLLUM GEN. NOV.
BASED ON CYSTOCARP DEVELOPMENT
AND MOLECULAR DATA

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The new genus *Neoharaldiophyllum* J.C. Kang *et* M.S. Kim belonging to the tribe Myriogrammeae, subfamily Phycodryioideae of the Delesseriaceae, is described. This new genus consists of four species, namely *Neoharaldiophyllum udoense* (M.S. Kim *et* J.C. Kang) J.C. Kang *et* M.S. Kim gen. *et* comb. nov. from Jeju Island Korea as the type species, *Neoharaldiophyllum nottii* (R.E. Norris *et* M.J. Wynne) J.C. Kang *et* M.S. Kim comb. nov. from the Hood Canal, Mason County, Washington, USA, *Neoharaldiophyllum mirabile* (Kylin) J.C. Kang *et* M.S. Kim comb. nov. from Canoe Island, San Juan County, Washington, USA, and *Neoharaldiophyllum erosum* (Harvey) J.C. Kang *et* M.S. Kim comb. nov. from Garden Island, Western Australia. The morphological traits of the new genus are very similar to the genera *Haraldiophyllum* and *Myriogramme* in terms of the vegetative and reproductive structures. There are differences among the three genera in the developmental patterns of the carposporophyte: the primary gonimoblast cells of *Neoharaldiophyllum* are prostrate on the floor cells of the cavity of the cystocarp and fuse together secondarily, whereas in the two other genera they remain free without a secondary incorporation with the floor cells; the

carposporangia of *Myriogramme* are borne in short chains terminating the gonimoblast filaments, while those in the other two genera are borne as solitary structures. The molecular phylogenies based on *rbcL* and LSU sequences strongly support the significance of the developmental patterns of the carposporophyte and support the separation of *Neoharaldiophyllum* from *Haraldiophyllum* in the tribe Myriogrammeae.

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MID- TO LATE- HOLOCENE HISTORY
OF LONG-TERM AND EPISODIC
MARINE ENVIRONMENTAL CHANGES
OF THE SOUTHERN BALTIC SEA –
MULTIPROXY APPROACH

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Sediments of two long gravity cores from the Gulf of Gdańsk and Bornholm Basin were studied for their siliceous microfossil assemblages to detect mainly surface water temperature, salinity and productivity changes during the mid and late Holocene. Cores were dated by AM-S14C. Simultaneously, other sediment proxies, including foraminiferal assemblages, XRF scanning and various geochemistry analyses, were used to provide a comprehensive reconstruction of ecosystem changes. These integrated studies were carried out within the framework of the “ClimLink” project. The 6.3-m long gravity core from the Gdańsk Deep and 8-m core from the Bornholm Basin cover, respectively, the last 6,700 and 7,000 cal. yr BP of the Baltic Sea’s history. Major changes in siliceous microfossil assemblages (diatoms, *Chaetoceros* resting spores, Chrysophycean cysts, *Ebria tripartita*, silicoflagellates) preserved in the sediments correspond with marine environmental conditions during the Littorina-Post-Littorina-Sea stages. Diatom species composition was influenced by regional factors such as riverine water input, as well as changes affecting almost the whole Baltic ecosystem, such as events

of strong Atlantic water inflow. Also, appearance of foraminifera in the sediment record confirmed a strong effect of saline and well oxygenated water inflows from the Atlantic Ocean on the Baltic Sea ecosystem, which might be linked to the major climate transitions over the last 7 millennia. In two parallel cores, data from the youngest sediments showed a decrease in diatom species richness. This suggests higher water turbidity caused by progressive eutrophication, therefore conditions have become favorable for small, planktonic, eutrophic/mesotrophic taxa so-called “anthropogenic assemblages”.

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**PEDIASTRUM SENSU LATO
(CHLOROPHYCEAE, SPHAEROPLEALES)
FROM THE ISLAND OF SULAWESI
(INDONESIA): MORPHOLOGICAL
AND MOLECULAR STUDY**

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Five strains of *Pediastrum sensu lato* have been studied. Two of them belong to *Pediastrum duplex* Meyen, two strains belong to *Stauridium tetras* (Ehrenberg) E.Hegewald and one strain belongs to *Monactinus simplex* (Meyen) Corda. Strains were isolated from the water bodies of the Sulawesi island. All strains were examined using LM and SEM. ITS rDNA was sequenced and compared with sequences from the GenBank database. A strain isolated from the ancient lake Matano and morphologically identified as *S. tetras* probably represents a species new to science. *Stauridium tetras* is a common and variable species with several varieties. It is very likely that our new species represents one of the described varieties, which should be raised to species rank in this case.

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**GRATOL-RIGHT: PROGRESS
AND CHALLENGES IN CHAROPHYTIC
GREEN ALGAE**

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The Green Algal Tree of Life project (GrAToL) has focused on resolving relationships among the phylogenetic breadth of green algae and land plants, including many previously unstudied taxa, by compiling and analyzing large amounts of genomic data. As diverse primary producers with essential ecological roles in oceans, fresh water, desert soil crusts, tropical and temperate forests, thermal hot springs and hypersaline ponds, green algae are important players in the realm of global ecosystem services and in their role as living relatives of land plants, evolutionary precursors and ecological partners in the evolution of the land animals. This poster highlights progress and challenges in resolving relationships among members of charophytic green algae, a paraphyletic assemblage of six small (one taxon) to large (thousands of taxa) clades that are part of the lineage that includes land plants. This poster summarizes some of the highlights that GrAToL has produced for this lineage, which we call GrAToL-Right, given its conventional placement on the primary split between the two major clades of green algae (see Lewis *et al.* companion poster on GrAToL-Left). In the context of a phylogeny of the charophytic algae *sensu lato*, we highlight the monophyly of most of the six charophytic algal clades (in contrast to the dramatic dismemberment of some chlorophytic clades (Lewis *et al.* poster), as well as the discovery of new species, plastid genomic architecture, and functional transcriptomics. We also note the ambiguous positioning of lineages of unicellular taxa that have been placed in a basal polytomy in the overall phylogeny of green algae.

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**TEMPORAL ACCLIMATION
OF NANNOCHLOROPSIS GADITANA
CCMP526 IN RESPONSE TO HYPER-
OSMOLARITY**

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Evaporation from open culture ponds and raceways can subject algae to salinity stress, and this is exacerbated by global warming. However, relatively few studies have focused on the effect of salinity on microalgae and their acclimation strategies. We investigated the effect of salinity on a marine microalga, *Nannochloropsis gaditana*, which is of industrial significance because of its high lipid-accumulating capability. Both short-term and long-term effects of salinity was studied across various salinities (37.5, 55, 70 and 100 PSU – Practical Salinity Unit). Salinity above 55 PSU suppressed cell growth. The specific growth rate was significantly reduced, and doubling time increased, at 100 PSU. Maximal quantum yield (Fv/Fm) of cells grown at high salt concentrations were lower than for cells grown in normal sea water (37.5 PSU). The photosynthetic parameters were further evaluated by measuring the maximum relative electron transport rate (rETRmax), light harvesting efficiency (α) and light saturation point for photosynthesis (Ik). rETRmax and Ik values were severely affected at high salinity conditions, though α was only affected in short term responses. However, there was no significant difference in Fv/Fm, rETRmax and Ik values between the cells grown at 37.5 PSU and 55 PSU. This indicates that the cells at 55 PSU could be employing photosynthesis as a potential strategy to overcome salinity. The chlorophyll a and carotenoid contents per cell increased at 100 PSU, suggesting the possible role of reactive oxygen species (ROS) quenching by carotenoids. Further, total carbohydrate per cell increased ~2 fold after 24 hr, which is consistent with previous findings that high salt condition induces osmolyte production to counter osmotic shock. Our findings indicate a possible mechanism of acclimation to salinity, opening up new frontiers for osmolytes in pharmacological and cosmological applications.

PRODUCTION AND GROWTH OF *KAPPAPHYCUS ALVAREZII* UNDER DIFFERENT CULTIVATION AREA IN EASTERN INDONESIA

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Production of *Kappaphycis alvarezii* in various cultivation areas depend on external and internal conditions. Environmental conditions are an important factor in the growth rate of *K. alvarezii*. This research tries to explain production of *K. alvarezii* in various cultivation areas at East Indonesia. During our research, highest production was occurring in Lakorua Station and Doda Bahari Station and low production occurs in Lakeba Station. From 5 kg, 7 kg and 9 kg in first weight, after 40 days was becomes 35.96, 37.54, and 39.96 kg in Lakeba Station and 32.58, 36.52, 39.18 kg, in Doda Bahari Station and 22.68, 23.74, 29.98 kg in Lakbe Station. Average of Specific Growth Rate (SGR) was high in Lakorou Station with 5.9, 5.2, 5.0 in 5, 7 and 9 kg in first weight, respectively. Average of current velocity at Doda Bahari station during July to September was high 0.065–0.097 m/sec. Range of nitrate and phosphate concentration during July to September was high in Doda Bahari station were 0.045–0.071 mg/L and 0.0047–0.0075 mg/L respectively. High growth rate of *K. alvarezii* addressed to the current velocity, nitrate and phosphate concentration.

PHYLOGEOGRAPHY OF BASAL TAXA OF KELPS

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The geographical origin of Laminariales, or so-called kelps, was controversial before the application of molecular phylogeny. The families having terete sporophytes such as Chordaceae and Pseudochordaceae, as well as Tilopteridaceae having foliose sporophytes but lacking meristematic zones,

have been considered as basal taxa, and there have been discussions on the origin of the order from either the North Atlantic or the North Pacific Oceans. Molecular phylogeny suggested that Chordaceae and Pseudochordaceae (and more recently described Akkesiphycaceae) were sister taxa of derived laminarialean members having foliose sporophytes with meristematic zones, such as Laminariaceae, whereas Tilopteridaceae was shown to be phylogenetically distant. Because Akkesiphycaceae/Pseudochordaceae have been only known from the NW Pacific, and basal members of Chorda are also distributed in the NW Pacific, we have concluded that Laminariales originated from the NW Pacific. Although the phylogenetic link between those basal taxa and derived families is still not clear, the new discovery in the Aleutian Islands of *Aureophycus*, which has rather simple sporophyte morphology lacking any structures related to mucilage secretion and forming sori on the discoid holdfast, may provide clues for answering the question. The present talk discusses updated phylogeography, especially on the dispersal of the basal members of Laminariales to the Northeastern Pacific and the Atlantic.

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ALGONONICS: INTEGRATING NANODOTS TO ENHANCE ALGAL BIOFUELS DEVELOPMENT

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Algal systems have the potential to alter the biofuels landscape by enabling the direct capture of the sun and its conversion to valuable biofuels and other co-products. However, there are a number of bottlenecks that limit algal capabilities and have slowed the development and large scale application of microalgae. One is their limited light-capture bandwidth which can be restrictive and is based on the natural photosynthetic capture mechanism. Improvements in light capture by microalgae has the potential to get the best of the natural capability in conversion of light to chemical entities. The capabilities of nanomaterials devices to capture available light across a wider spectrum is an emerging field. The impact of enhancing light capture in microalgae was studied by supplementing *Chlamydomonas reinhardtii* cc503 with Green Graphene Quantum Dots (GGQD). Intake of GGQDs within the microalgae was determined by TEM. Biological compatibility was observed by non-toxic nature of GGQDs. There were no morphological or chemical changes except for photo-quenching of GGQDs. Despite photo-quenching,

cells showed growth and an increase in lipid production. Furthermore, an investigation was done for lipid and Fatty Acid Methyl Ester (FAME) upsurge and we found that an increase in the concentration of H₂O₂ might have caused the enrichment of lipids and FAME. The results indicate specific relation between GGQDs intake and impact on electron gain or loss for fluorescence quenching activity, improving bio-compounds production.

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COMPARISON OF C-PHYCOCYANIN PURIFICATION IN DIFFERENT CHROMATOGRAPHIC MEDIA

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Phycocyanin (PC) is a major phycobiliprotein produced by cyanobacteria, rhodophytes, and cryptophytes. Phycocyanin has been the subject of active research because of the potential for its application as a natural pigment (food colorant, cosmetic), its therapeutic properties including anti-inflammatory, neuroprotective, antioxidant, anti-cancer activities and its fluorescence properties. In the present study PC was extracted with 1.5% calcium chloride and purified by a three-step sequential method consisting of fractional ammonium sulfate precipitation, size exclusion chromatography (using Sephadex G-25 and Sephadex G-50) and ion exchange column chromatography (Using DEAE-Sephadex and DEAE-Cellulose). The purity ratio of eluted fractions of the two different gel and ion exchange media was compared. PC purity was assayed as a function of the purity ratio, defined as an absorbance ratio A_{620}/A_{280} . The fractions collected through Sephadex G-50 and DEAE-Cellulose had a purity ratio of 3.8, whereas the fractions collected through Sephadex G-25 and DEAE-Sephadex exhibited reactive grade purity of 4.3 (A_{620}/A_{280} ratio greater than 3.9). SDS-PAGE of pure C-phycocyanin yielded two bands corresponding to α and β subunits. The estimated molecular weights of α and β phycocyanin subunits from *Spirulina platensis* were 18 and 22 kDa, respectively.

ENHANCEMENT OF FUCOXANTHIN CONTENT OF PHAEODACTYLUM TRICORNUTUM

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Fucoxanthin is a major carotenoid, present in both macro and microalgae. Fucoxanthin has been found to have remarkable biological properties such as antioxidant, anti-inflammatory antiobesity, antitumor, antidiabetic and hepatoprotective activities. In this study, response surface methodology (RSM) based on central composite design (CCD) was applied to evaluate optimal culture parameters (temperature, light irradiance and agitation speed) to enhance fucoxanthin content of *Phaeodactylum tricorutum*. The individual and interactive effects of temperature, light irradiance and agitation speed on fucoxanthin production were also evaluated. The 3D response surface plot and the contour plot derived from the mathematical models were applied, and they showed that a temperature of 15°C, light irradiance of 55 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ and agitation speed of 130 rpm were the optimal conditions. The predicted maximum fucoxanthin content by RSM was 7.9 mg g⁻¹, whereas maximum fucoxanthin content of 10.2 mg g⁻¹ was obtained in the validation experiments under optimized conditions after 12 days cultivation. The adequately high *R*-Squared value (96.9%) indicated the statistical significance of the model.

PRELIMINARY RESULTS ON USE OF DIATOMS FOR BIOMONITORING RIVERS IN THE CENTRAL ALBORZ, IRAN

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Diatoms are useful for detecting changes in waterbodies, including rivers, and assessing water quality due to the diatom communities' specific sensitivities to a variety of ecological conditions. In meeting the EU Water framework Directive in the determining pollution level of water, different diatom indices have been developed and are used in America and European countries. However, the use of diatoms in biomonitoring programs in Iran is just beginning. This research presents biomonitoring work on the basis of diatom indices in the Karaj river, which flows south from the Central Alborz Mountains in north-central Iran. This is only the second study using diatom indices to assess the water quality of rivers in Iran. Epilithic samples were collected from six stations along the river between March 2011 and April 2012 and 128 diatom taxa were identified. Relative abundance of species were calculated from 300-valve counts of each sample. Evaluation of species indices and data analyses were accomplished using Omnidia and Spss 16 software. The relationship between three diatom indices (pollution sensitivity index, IPS; Descy/Coste diatom index, CEE; trophic diatom index, TDI) and environmental parameters was examined in four months (July, August, October, and March). According to IPS and CEE indices, the quality of water fell into the category of good to high quality. With the modified TDI index, the quality of the water was determined as moderate to high in different stations during the year. According to van Dam's trophic index, the river was classified as oligo-mesotrophic to meso-eutrophic seasonally. However, diatom indices showed weak correlation with measured physico-chemical variables. IPS was correlated with specific conductance and dissolved phosphorus concentrations in August, and CEE was correlated with SO₄ and Si in March, October and August. Complementary work should be done on other rivers of Iran to explore the usefulness of diatom indices for comparing water quality among waterbodies of the country.

TWO UNKNOWN SPECIES OF SIMONSENIA (BACILLARIOPHYCEAE) FROM TIDAL FLATS ON THE WEST COAST OF KOREA

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Simonsenia Lange-Bertalot is a diatom genus with a distinctive morphology belonging to the family Bacillariaceae Ehrenberg. Only four species and one subspecies have been reported to date and published records suggest a worldwide distribution of some of those species. Here we present the results of our research on two previously unknown species of *Simonsenia* from the tidal flats of Shinan and Taean in Korea. *Simonsenia* sp. 1 from Shinan was sampled in June 2016. The valves are 11–12 μm long and 1.9–2.1 μm wide, with a fibula density of 14 in 10 μm . Transapical ribs and striae are not resolved. A row of poroids delineating a boundary line of fibulae distinguishes this species from established *Simonsenia* species. The strain of *Simonsenia* sp. 2 from Taean was isolated into culture in December 2016 and was subject to SEM examination and molecular phylogenetic analysis. The valves are 12.8 μm long and 1.9 μm wide. The density of transapical ribs, fenestrae and fibulae are 22–23, 24–25 and 10–12 in 10 μm , respectively. These dimensions and fine structure are similar to those of *S. delognei*, but the hymenate occlusions of areolae differentiate this species from *S. delognei*. A molecular phylogeny based on sequences of the chloroplast-encoded *rbcL* gene of *Simonsenia* sp. 2 showed affinity with *S. aveniformis* and the two species fell in the same clade.

PATHOGEN-SPECIFIC GENE REGULATION IN THE RED ALGA PYROPIA TENERA AGAINST THE THREE MOST COMMON DISEASES IN SEA FARMS

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Outbreaks of diseases devastate *Pyropia* aquaculture farms every year causing US\$ multimillion dollar loss. The three most common diseases are *Olpidiopsis* blight and red-rot disease caused by oomycete pathogens and green-spot disease caused by PyroV1 virus. We hypothesized that a basic genetic profile of molecular defences will be revealed by comparing and analyzing the genetic response of *Pyropia tenera* to these three pathogens. RNAs isolated from infected thalli were hybridized onto an oligochip containing 15,115 primers designed from *P. tenera* ESTs. Microarray profiles of the three diseases were compared and interpreted together with histochemical observation. Massive amounts of reactive oxygen species (ROS) were accumulated in *P. tenera* cells exposed to oomycete pathogens. Heat shock genes and serine proteases were the most highly upregulated genes in all infection experiments. Genes involved in RNA metabolism, ribosomal proteins and antioxidant metabolism were also highly upregulated. Genetic profiles of *P. tenera* in response to pathogens were most similar between the two biotrophic pathogens, *Olpidiopsis pyropiae* and PyroV1 virus. A group of plant R-gene homologues were specifically regulated against each pathogen. Our results suggested that disease resistance of *P. tenera* consists of a constitutive defence using ROS signals and a genetic toolkit against specific pathogens.

TUNING SEA URCHIN DENSITY FOR RECOVERY AND MAINTENANCE OF KELP BEDS IN THE BARREN SUBTIDAL HABITAT

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Urchin barrens are common in the east coast of Korea, comprising about 60% of all rocky shallow subtidal areas. The average density of urchins is 7.8 individuals/m² in the completely barren sites. Experimental manipulation of sea urchin density was conducted on nine natural bed rocks in barren condition, each separated by sandy barriers in between. Three experimental conditions were applied: total urchin removal, “half”-removal, and no-removal as a control. At all removal sites, the macroalgal community was recovered and in good shape for three consecutive spring seasons (2015–2017), whereas the barren state continued at the no-removal control sites. For the “half”-removal condition, we started with 4.0 individuals/m², then switched to 2.5 individuals/m² to achieve an optimal density level, which may be necessary for the maintenance of kelp forests. In another part of the study, we reintroduced sea urchins (7.8 individuals/m²) to the ‘healthy’ kelp beds to see how fast the kelp bed deforested and concluded that it took 11 months. This study provides a valuable guideline in controlling urchin density for community shift from barrens to forest and its maintenance.

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ENHANCEMENT OF ZEAXANTHIN PRODUCTION OF DUNALIELLA TERTIOLECTA BY RANDOM MUTAGENESIS AND OPTIMIZATION OF CULTURE CONDITIONS

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Zeaxanthin is a xanthophyll pigment that is regarded as one of the best carotenoids for the prevention and treatment of degenerative diseases. In the worldwide natural products market, consumers prefer pigments that have been produced from biological sources. However, it is hard to earn the perfect wild-type for zeaxanthin production, hence, introduction of non-GMO method is necessary. In this study, a *Dunaliella tertiolecta* strain that has 10–15% higher cellular zeaxanthin content than the parent strain (*zea1*), was obtained by random mutagenesis using ethyl methanesulfonate (EMS) as a mutagen. This mutant, *mp3*, was grown under various salinities and light intensities to optimize culture conditions for zeaxanthin production. The highest daily zeaxanthin productivity was observed at 0.6 M NaCl culture medium and mid-light condition

(140–160 µE). To measure the maximal yield of zeaxanthin from *mp3*, we conducted repeated fed-batch culture on 200-mL culture medium on 500-mL flask. The maximal zeaxanthin yield was 8 mg/L from *mp3* strain, which was about 20% higher than *zea1* (6.5 mg/L). These results suggest that random mutagenesis with EMS is useful for generating *D. tertiolecta* strains with increased zeaxanthin content and also suggest optimal culture conditions for the enhancement of biomass and zeaxanthin production of our mutant strain.

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SURVEYS ON HIGH-TEMPERATURE RESISTANT NATURAL POPULATIONS OF PYROPIA SPECIES IN KOREA

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Species of *Pyropia* are commercially important edible red algae in Korea, regarded as a low energy density food, rich in dietary fiber and containing high contents of vitamins, minerals, and antioxidants. In Korea, the yearly production of *Pyropia* is ca. 250,000–400,000 ton yr⁻¹ and worth up to \$2 billion and farming proceeds from October to March. The *Pyropia* gametophytes (i.e. the blade stage) are not grown in the summer months in the sea farms as they prefer colder seawater and air temperatures and deteriorate quickly at temperatures exceeding 20°C. Due to increasing domestic consumption and export, more demands for *Pyropia* production have arisen during the last decade. In order to find a high-temperature resistant strain of *Pyropia*, which can tolerate seawater temperature over 20°C, we surveyed the western and southern coasts of Korea and found several natural populations of the gametophytes growing in the warmer months. Carpospores released from the blades in the laboratory were settled on suspended dead oyster shells and the conchocelis phase of two *Pyropia* species was obtained. The conchospores released from the mature sporophytes developed into blades, completing the life cycle in 1 year. The rcbL and SSU rRNA gene sequencing results showed that our temperature-resistant strains belong to the clade including *P. haitanensis* and *P. dentata* from Japan,

China and Taiwan, but are different from the strains recorded in those countries. Our molecular-phylogenetic analyses showed that sequences in each group (i.e. 'haitanensis' and 'dentata') were very heterogeneous, for example, for the *rbcL* gene different isolates of *P. haitanensis* and *P. dentata* had 95.8–100% and 93.3–100% identity, respectively. In SSU rRNA gene, different isolates of *P. haitanensis* and *P. dentata* had 74.7–99.7% and 82.7–78.2% identity, respectively. Even isolates of the same species from the same country varied significantly suggesting the locality of each strain is overlapping in Far Eastern countries.

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THE ITS2 GENETIC POLYMORPHISMS WITHIN PSEUDO-NITZSCHIA PUNGENS (BACILLARIOPHYCEAE) RESULTED FROM THE SEXUAL REPRODUCTION

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The bio- and genetic diversity of species could prevent the extinction from natural selection and provide the primary abilities to adapt to the various habitats. The pennate diatom, *Pseudo-nitzschia pungens* is cosmopolitan and regarded as a common component of marine phytoplankton. The genetic diversity and distinct three genotypes (clades) of *P. pungens* have been well-studied. However, experimental proof of genetic polymorphism in this species has not been provided until now. We analyzed morphological features using LM and TEM images, and examined the ITS2 rRNA gene using genetically different (clade I and III) *P. pungens* strains, two parental and its two offspring, through cloning PCR. There were no morphological differences between parental and offspring strains, however the offspring strains showed recombinant sequences types in ITS2 region. The ITS2 sequences of offspring were composed by the sequences of clade I or III. Additionally, high ratios (38.6 and 65.1%) of single nucleotide polymorphisms (SNPs) were observed in the ITS2 region of offspring strains. Our results imply that the genetic polymorphism phenomenon, such as genetic recombination and SNP generation, resulting from sexual-reproduction should

contribute to maintain the genetic diversity and to produce the new genotypes within this species. Furthermore, the diverse hybrid types might provide adaptive strategies to various natural environments and may even be the source of new recombinant species in the future.

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MORPHOLOGICAL AND MOLECULAR STUDIES OF THE MYSTERIOUS DIATOM PROSCHKINIA FROM KOREAN MUD FLATS RESOLVE ITS PHYLOGENETIC POSITION

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Only seven species of the diatom genus *Proschkinia* Karayeva have been reported since the genus was described and removed from *Navicula* sensu lato. We have recently identified five species of *Proschkinia* from the two mudflats of Beopsan-ri and Pado-ri of the Yellow Sea coast of Korea. The *Proschkinia* strains were isolated from mud and seaweeds and are maintained in culture. Their morphology was studied by means of scanning electron microscopy (SEM), which revealed that each *Proschkinia* species possesses a peculiar fistula distinguishing them from other taxa, with raised wart-like structures covering internal opening of fistula. The fistula of *Proschkinia* sp. 1 has slightly round, long circular bundles of warts. *Proschkinia* sp. 2, the smallest species, with slightly rounded raphe endings and somewhat linear stria, bears fistula with only one raised wart. In *Proschkinia* sp. 3, which has somewhat sharp raphe endings, the fistula is covered with two warts. *Proschkinia* sp. 4 is lanceolate with fistula possessing three warts. The longest taxon, *Proschkinia* sp. 5, has a fistula similar to that of *Proschkinia* sp. 1, but the ultrastructure of the warts is clearly different. In this study we also present for the first time a molecular phylogeny, based on the chloroplast-encoded *rbcL* gene, of taxa belonging in *Proschkinia*. Our results

show that our five *Proschikina* species are monophyletic and are sister to a clade containing *Fistulifera* Lange-Bertalot, which also possess fistula. This implies that the fistula may be a shared character between the genera and plays an important role in evolution of Proshkiniaceae. We propose that the fistula is the character by which the Proshkiniaceae can be separated from the Stauroneidaceae.

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EFFECT OF SEX PHEROMONE ON THE SIGN OF PHOTOTAXIS IN BROWN ALGAL MALE GAMETES

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The behaviors of microorganisms, including brown algal swimmers are the result of a delicate balance between positive and negative phototaxis so called “sign of phototaxis”. Freshly released gametes of anisogamous brown alga *Mutimo cylindricus* swim toward the light direction (positive phototaxis) for several hours, and after that they start to accumulate in the opposite side to the light direction (negative phototaxis).

In this study, we found the sex pheromone from female gametes to affect the sign of phototaxis of male gametes. Most of male gametes of *M. cylindricus* suddenly switched the phototactic sign (positive to negative) by the reception of sex pheromone from female gametes. The three main compounds (multifidene, auctantene and ectocarpene) of sex pheromone were already identified from female gametes of *Cutleria multifida*, which is phylogenetically close to *M. cylindricus*. To understand the phenomenon more precisely, we analyzed the effect of synthesized ectocarpene on the sign of phototaxis in both male and female gametes in *M. cylindricus*. At the concentration of 3.25×10^{-5} M ectocarpene, male gametes showed positive phototaxis as well as the condition without ectocarpene. At the concentration higher than 6.5×10^{-5} M ectocarpene, many of male gametes showed negative phototaxis. In contrast, female gametes

showed positive phototaxis in all conditions. Since female gametes show negative phototaxis to settle on the substratum prior to male gametes and secrete sex pheromone, these results suggest that switching phototactic sign (positive to negative) in male gametes in the presence of the sex pheromone contribute to the efficient fertilization by increasing the opportunity to encounter female gametes.

We previously reported that lowering extracellular calcium ion concentration induced the change of phototactic sign (positive to negative). Taken together, we propose that the change of phototactic sign of male gametes by sex pheromone can be regulated by the alteration of intracellular calcium ion concentrations.

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DECIPHERING THE ROLE OF THE (UN) CULTIVABLE MICROBIOME DURING FRESHWATER ACCLIMATION IN THE BROWN MACROALGA *ECTOCARPUS SUBULATUS*

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The freshwater strain of *Ectocarpus subulatus* is unique for its ability to grow in fresh water, a physiological trait that has been shown to depend on interactions with symbiotic bacteria. We aim to elucidate the functional mechanisms involved in the acclimation of the algal holobiont to fresh water, emphasizing the metabolic interactions between the alga and the microbiome. To this aim, we first sought to create a simplified and controllable microecosystem via both the cultivation of bacterial partners and production of axenic algal filaments. Bacteria were isolated using direct-plating and dilution-to-extinction techniques, with both chemically defined and undefined low-nutrient culture media. Ground algae, algal culture medium, and cell

wall extracts were used as inocula. This resulted in the isolation of 376 strains, covering 45 bacterial species, phylogenetically dispersed into the Betaproteobacteria (33%), Alphaproteobacteria (35%), Gammaproteobacteria (21%), Firmicutes (3%), Bacteroidetes (4%), and Actinobacteria (4%). Antibiotic-treated algal filaments were then grown in co-culture with the cultivable bacteria: neither single bacterial species nor small artificial communities were able to stimulate algal growth in fresh water, suggesting an important role of the “uncultivable” microbiome during acclimation to freshwater. In order to generate hypotheses about the metabolic interactions between the alga and the uncultivable microbiome, we chose to work with algal holobionts with reduced microbiomes resulting from different (mild) antibiotic-treatments. These holobionts differ in their microbiome composition and in their capacity to grow in fresh water and they will be investigated using metabolomics and transcriptomics during the change in salinity. The implementation of this integrated ‘meta-omics’ approach as an alternative to the simplified co-culture system is in progress, and the generated data will help us to investigate to what extent bacterial metabolism complements that of the alga during fresh water acclimation.

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DIVERSITY AND TAXONOMY OF FRESHWATER AND SUBAERIAL MEMBERS OF ULVALES/ULOTRICHALES (ULVOPHYCEAE, CHLOROPHYTA)

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We focus on the order Ulvales/Ulotrichales. This order includes a number of algae living in a broad variety of habitats, in terrestrial as well as marine or freshwater ecosystems. This aspect together with prevailing uncertainty in taxonomy of certain species may be some of the reasons why molecular and phylogenetic information about this group are still limited although a lot of ecophysiological or morphological studies have already been carried out. In this research we gather strains from foreign algal culture collections and also isolate strains from natural localities

within the Czech Republic, Sardinia and particular type localities. We apply a complex approach using morphological as well as molecular methods to address some burning questions that still remain unanswered. Our main goal is to discover the real diversity of the freshwater and subaerial members of Ulvales/Ulotrichales and to elucidate the true relationships in some problematic genera (*Dilabifilum*, *Pseudendoconium*).

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A WORKFLOW FOR LARGE-SCALE PERMANENT SLIDE IMAGING AND IMAGE ANALYSIS FOR DIATOM MORPHOMETRICS

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Light microscopy analysis of diatom frustules is widely used in basic and applied research, notably taxonomy, morphometrics, water quality monitoring and paleo-environmental studies. Building on automated imaging solutions developed for medical/histological microscopy, we can now implement substantial parts of an automated diatom imaging workflow. Our methods resemble those drafted by the ADIAC (Automated Diatom Identification And Classification) project, and combine a commercial slide-scanning microscope with our automated diatom image analysis software SHERPA for measuring a broad range of morphometric features from individual frustules mounted on permanent slides. Although a fully automated process enabling routine diatom counting is still far from becoming reality, user intervention is minimized by extensive automation and internal quality control of the results. Furthermore care was taken to allow the user to stay in control of the most critical steps (exact segmentation of valve outlines and selection of objects of interest) using interactive functions for reviewing and revising results. We successfully applied our methods to several projects by finding, selecting and measuring up to several ten thousand diatom valves (presented at the poster session), and present our workflow in the hope that it can facilitate research on diatom morphometry.

HIGH THROUGHPUT (SEMI-)AUTOMATED DIATOM MORPHOMETRICS WITH SHERPA: IN AND BELOW THE SOUTHERN OCEAN

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Our project is aimed at bringing automated microscopy and image processing methods into routine diatom analysis from the water column, sediment traps and sediment cores in the Southern Ocean. We combined a Metafer slide scanning system with SHERPA, our image analysis software developed specifically for diatom images, to characterize morphometric variation within and among diatom species. This poster gives an overview of the established methodology (presented in detail by the according talk), and its application to three studies in a paleoceanographic, a life cycle and a taxonomic context.

REPRODUCTIVE ISOLATION VIA SEX PHEROMONES IN CLOSTERIUM PERACEROSUM-STRIGOSUM-LITTORALE COMPLEX

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Closterium is a genus of unicellular, isogamous, charophycean algae that share a close phylogenetic relationship with land plants. Heterothallic strains of the *Closterium peracerosum-strigosum-littorale* (*C. psl.*) complex consist of two sexes: mating-type plus (mt+) and mating-type minus (mt-). The conjugation process involves the pairing between mt+ and mt-, release of protoplasts from paired cells, and zygospore formation. The mt+ and mt- cells recognize each other through chemical communication by mating-type-specific sex pheromones, protoplast-release-inducing protein (PR-IP) inducer and PR-IP. At least six reproductively isolated mating groups have been observed among strains of the heterothallic *C. psl.* complex.

The objective of this study is to identify the barrier causing reproductive isolation. We focused on the closely related mating groups: I-E and G. When cells of mating group I-E and G were mixed, no zygospore formation was observed. However, some lone protoplast-releasing cells without pairing were observed between mt+ cells of I-E and mt- cells of G. The PR-IP partially purified from I-E could induce the lone protoplast-releasing of mt- cells of G, although the effect was lower than the effect against mt- cells of I-E. These results indicate that PR-IP is produced from mt+ cells of I-E through the sexual interaction with mt+ cells of G and exerts an effect against mt- cells of G. It is also probable that PR-IP Inducer produced by mt- cells of G is active against mt+ cells of I-E. The PR-IP partially purified from G also showed a protoplast-releasing activity against mt-cells of both I-E and G. However, the required concentration was apparently high, compared with the PR-IP from I-E. This might be a critical point of reproductive isolation between the two mating groups.

When mt- cells and mt+ cells of mating groups I-E and G were simultaneously mixed, zygote formation of mating group G was inhibited, but no inhibition was observed in group I-E. From these results, it was suspected that the mating group G suffered reproductive interference from mating group I-E, which may be due to the excessive action of PR-IP of group I-E.

FRESHWATER DIATOM BIOGEOGRAPHY: FROM 'EVERYTHING IS EVERYWHERE' TO ENDEMISM AND BEYOND

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Studies on the distribution of species, genera and families of freshwater diatoms are reviewed under the title of "biogeography". Starting from the middle of the 19th century with Ehrenberg, and continuing through the early and mid 20th century, the existence of endemic taxa and floras, and explanations to describe the distributions over time and space, were advanced. This approach helped document the many geographically-limited species, but did not override prevailing dogma that "Everything is everywhere but Ecology selects" borrowed from the bacteriological literature. Later in the 20th century a renewed interest and emphasis by taxonomists and systematists in floristics and monographic revisions helped to further document the

occurrence of endemic taxa. While many examples of endemics were documented, few patterns have been described and fewer explained.

Alternatively, and relatively much less frequent, has been the documentation of monophyletic groups and the resulting patterns over space and time. This “phylogeographic” approach combines the power of phylogenetic relationships with temporal and/or spatial data. Currently, these types of studies offer conflicting evidence for endemism and interpretable patterns.

Lack of a phylogenetic perspective in revisionary studies of diatoms is one roadblock to employing a phylogeographic approach to understanding the incredible diversity and levels of endemism found amongst the freshwater diatoms. Another may very well be that a limited, conservative taxonomy of freshwater diatoms at the level of genus (let alone other levels of taxonomic hierarchy) has obscured distributional patterns.

The studies of freshwater diatom biogeography and phylogeography are reviewed, including examples from the tropics, islands and Antarctica. Recent work in Southeast Asia is highlighted; once thought to comprised of cosmopolitan taxa the region is shown to harbor many endemic species and genera. Future work remains to match patterns with explanations of history for the freshwater.

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BEYOND NAMES: DEVELOPING A NAMES DATABASE AND OTHER APPLICATIONS FOR DIATOMS

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Names are an important reference for all aspects of Biology, Paleontology, Medicine and many other applications. Nomenclatural databases have been developed to document and identify names that have already been established, as well as when, where and by whom they have been proposed and published.

There has been a long history of catalogues of names representing the great diversity of diatoms. Some of these catalogues were limited in coverage and impact, others have been more complete, widely distributed and widely cited. The catalogue of VanLandingham, as well as the Index Nominum Algarum and the New Species File at ANSP were collaboratively integrated into a single database and made available on line and presented by Fourtanier and Kociolek as the Catalogue of Diatom Names. In its first iteration, the Catalogue of Diatom Names contained 64,000 names derived from over 12,000 references. It debuted in

2005, and in 2010 it was accessed by over 30,000 unique IP addresses and over 5,000,000 downloads. The database grew until 2011, but ceased to be updated since.

A new collaboration between an international group of editors has been formed, and working with VLIZ to produce a new, reliable resource for diatom names called DiatomBase (<http://www.DiatomBase.org>). This project takes the Catalogue of Diatom Names and integrates with the VLIZ Aphia database structure with names from Algaebase, to create a resource for diatom nomenclature and information. DiatomBase will also support a number of nomenclatural and other informatics efforts, as well as bring together images of types and other curated images, as well as linking to other resources. The group of editors, 20 people from 14 countries, has enhanced the speed in which DiatomBase has been developed, and will be critical for the long-term sustainability of the project. DiatomBase will debut at the International Phycological Congress.

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CRYPTIC SPECIES DIVERSITY OF THE GENUS *HERPOSIPHONIA* (RHODOMELACEAE, RHODOPHYTA) FROM THE SOUTH-WESTERN COAST OF SPAIN

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In Spain, the genus *Herposiphonia* has been recorded as only two species, *H. secunda* (C. Agardh) Ambronn and *H. tenella* (C. Agardh) Ambronn. These species are causing considerable taxonomic confusion and controversy because of their morphological variations. *RbcL* and COI-5P genes are well established to delimit species boundaries in red algae. We collected *Herposiphonia* specimens from south-western Spain and confirmed three species based on morphological difference and *rbcL* molecular analyses. Two species are identified as *H. secunda* by having d/i branching pattern and *H. tenella* by d/d/d/i branching pattern with presence of vegetative trichoblast. The third species is similar to *H. nuda* Hollenberg, with slender (50–60 µm) and long (2–3 mm) determinate branches with absence of vegetative trichoblast. In COI-5P DNA barcoding analysis, we compared our samples with 13 Hawaiian, four Spanish and two Portuguese sequences from GenBank. The results showed that these sequences were separated into several independent clades. It is interesting that only one sequence

of our samples, which is similar to *H. nuda*, was matched to a Spanish one (KF648513). In conclusion, four species are distinguished from Spanish specimens rather than two. We need to investigate the phylogenetic relationships of Spanish *Herposiphonia* species from the Atlantic coast.

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HIDDEN DIVERSITY IN THE GENUS *PYROPIA* (BANGIOPHYCEAE, RHODOPHYTA) FROM KOREA BASED ON GENETIC AND MORPHOLOGICAL EVIDENCE

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The worldwide revision of the Bangiales transferred most of *Porphyra* species to the genus *Pyropia*. As the result of taxonomic study of *Pyropia* in Korea based on *rbcL* gene analysis and morphology, we confirmed ten species with three unidentified species, *Pyropia* sp. 1, *Pyropia* sp. 2 and *Pyropia* sp. 3. *Pyropia* sp. 1 is characterized by ruffled, linear shaped, purple in color and 35–40 µm thick thallus. *Pyropia* sp. 2 is a monoecious thallus having spermatangial patches at the upper part and zygotosporengia at the middle. *Pyropia* sp. 3 is dioecious with spermatangial patches formed at the half of side of thallus and it was collected at fast-flowing water in 10–15 m depth. The molecular analysis of *rbcL* gene demonstrated that three unidentified species are clearly distinguished from other species of *Pyropia* in the phylogenetic tree with 1.1–8.4% interspecific divergence. In conclusion, on the basis of molecular and morphological results, we propose three new cryptic species from Korea: *Pyropia ulleungensis* sp. nov. (as *Pyropia* sp. 1), *Pyropia vittata* sp. nov. (as *Pyropia* sp. 2) and *Pyropia sub-marina* sp. nov. (as *Pyropia* sp. 3).

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ENVIRONMENTAL DNA-METABARCODING AND LOCAL REFERENCE BARCODING REVEAL REMARKABLY HIGH SPECIES DIVERSITY IN THE PLANKTONIC DIATOM FAMILY CHAETOCEROTACEAE

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Assessment of morphological and molecular diversity in the planktonic diatom family Chaetocerotaceae (using isolation and cultivation) uncovered ca. 80 species, 44 of which were observed in the Gulf of Naples (GoN, Mediterranean Sea). Subsequently, we assessed the diversity in this family by means of high throughput sequencing (HTS) metabarcoding of 48 protist samples collected at the LTER MareChiara (GoN) between 2011 and 2013. As a metabarcode we used the V4 hypervariable region in the 18S rDNA. The 13.6M obtained eukaryotic contigs grouped into 615,142 haplotypes, of which 18,625 were assigned to Chaetocerotaceae (EPA and BLAST using chaetocerotacean reference barcodes). Following elimination of haplotypes with <3 contigs, the retained 650 haplotypes were aligned with the ca. 80 Chaetocerotacean reference barcodes to build an ML tree. This tree resolved 66 terminal clades assigned putatively to *Chaetoceros* species and ten to *Bacteriastrium*. Of these 76 clades: 39 were assigned to species known from the GoN (either previously described ones, or newly discovered ones still in need of description), seven to a species not known from the GoN, and the remaining 30 lacked a reference barcode. These species have probably been observed in the regular cell counts at the LTER, but are categorized as *Chaetoceros* spp. as these are difficult to distinguish in LM. Whilst gathering rDNA sequences of the chaetocerotacean strains, introns were encountered at 14 positions in the 18S rDNA alignment, *C. diversus* exhibiting nine introns. Fortunately, the V4-region, exhibited an intron in only two species and the V4 universal primer positions were fatally corrupted in only one species. Thus, HTS-meta-barcoding works well in detecting and discriminating among the members of this common and abundant family. Moreover, HTS metabarcoding results show that even in the taxonomically well-explored GoN a considerable percentage of the chaetocerotacean species is still to be described.

NOSTOCALES WERE NOT FAVORED BY INTENSIFIED NITROGEN LIMITATION

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Some argue that reducing nitrogen loads will favor N₂ fixing cyanobacteria and thus impair phytoplankton composition instead of lowering its biomass. Such long-term effects are not covered by bioassays or short-term monitoring. The polymictic lake Müggelsee (Berlin, Germany) has been comprehensively monitored since 1976. We analyzed this unique dataset to test the following hypotheses: In polymictic lakes, reductions in external nitrogen and phosphorus loads cause an immediate decline in N and a delayed decline in P concentrations, followed by intensified N limitation without increasing the share of N₂ fixing cyanobacteria. Since the 1980s, the external TP load declined by 68%. In-lake P concentrations declined with a delay of 5–10 years, with strong P remobilization from sediments in each summer. In contrast, the 79% decline in external TN load caused an immediate, parallel decrease in lake N concentrations. Periods of low N supply lasted longer, the N:P ratios declined. The total phytoplankton biomass correlated strongly with TP and TN in spring ($r^2 = 0.63$ each) but much less with TP ($r^2 = 0.12$) than TN ($r^2 = 0.78$) in summer. During summer, N₂ fixing cyanobacteria (mostly of the genera *Aphanizomenon* and *Anabaena*) contributed 68% to cyanobacteria and 45% to total phytoplankton biomass in the 1980s, but declined to 58% and 20%, respectively, in recent summers. Within the Nostocales, *Anabaena* species could only partly compensate for the almost complete decline of *Aphanizomenon* spp. The biomass-specific frequency of heterocysts increased at DIN (nitrate + ammonium) concentrations below 0.2 mg N / L but, due to the declining Nostocales biomass, the total number of heterocysts did not significantly change. Annual N₂ fixation always contributed less than 10% to total N load. We provide arguments for the prevalence of nitrogen limitation of polymictic lakes during summer and demonstrate expedience and sustainability of N load reductions to fight harmful algal blooms.

GENETIC DIVERSITY AND EVOLUTIONARY ADAPTATION IN POPULATIONS OF BLOOM FORMING BALTIC MICROALGAE

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Genetic diversity is an important requirement for populations to adapt to and persist in a changing environment. In the Baltic Sea, historic bottle-neck effects and harsh environmental conditions have been suggested to reduce genetic diversity and negatively affect the capacity of populations to withstand environmental changes. While this is well established for Baltic macroscopic algae, genetic diversity patterns of microalgae have long remained unknown. Here I present results of field and laboratory studies examining genetic structure and diversity of two bloom forming phytoplankton species in the Baltic Sea, *Skeletonema marinoi* and *Alexandrium ostenfeldii*. Population genetic analyses at different spatial and temporal scales across the North Sea – Baltic salinity gradient revealed that *S. marinoi* populations are genetically differentiated by physical barriers and adaptation to local salinity conditions. Though diversity is slightly reduced compared to neighboring marine populations, genotype diversity of brackish Baltic *Skeletonema* is remarkable and coupled to high phenotypic variation, which facilitates selection and stability of ecological functions under changed conditions in experiments. Temporal genetic differentiation observed during a *S. marinoi* spring bloom was correlated to decreased silica availability and likely reflects selection of low silica adapted individuals. Also local populations of the toxic dinoflagellate *A. ostenfeldii* show significant genetic differentiation among Baltic bloom sites, suggesting a strong anchoring effect of their ‘seed banks’. These contain high standing genetic variation as compared to active seasonal subpopulations which emphasizes their important role as genetic reservoirs in adaptation to environmental change.

HOW TO BUILD A GLASS HOUSE: INSIGHTS INTO THE BIOCHEMICAL MECHANISMS FOR SILICA BIOMINERALIZATION IN DIATOMS

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Diatoms are a large and ecologically important group of single-celled microalgae that contribute about 20% of the biological primary production on our planet. The most conspicuous feature of diatoms is their cell wall, which is composed of amorphous silica (~90 %) and tightly associated organic macromolecules (~10%). There are an estimated 100,000 diatom species, and each species produces a specific silica morphology that is genetically encoded. A hallmark of diatom silica is the highly porous light weight architecture that exhibits an extraordinarily high mechanical stability. The pores in the silica are hierarchically arranged and have diameters between ten to several hundreds of nanometers. Biosilica formation in diatoms is a bottom-up process that proceeds from monosilicic acid with remarkable speed (typically ~60 min) and under ambient conditions, thus by far exceeding the capabilities of present day materials engineering. How are diatoms able to rapidly produce such an amazing material with high fidelity? Here I will present the current knowledge about the molecular machinery for silica biomineralization in diatoms with a focus on the roles of proteins, insoluble organic matrices, and vesicles.

LA VIE DES ALGUES ROUGES: THE GENETICS AND EVOLUTIONARY ECOLOGY OF THE COLONIZATION OF NOVEL HABITATS IN HAPLOID-DIPLOID SEAWEEDES

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Baker's Law predicts uniparental reproduction will facilitate colonization success in novel habitats. While evidence supports this prediction among colonizing plants and animals, few studies have investigated shifts in reproductive mode in haplo-diplontic species, in which there are both prolonged haploid and diploid stages. In these life cycles, cross-fertilization between haploids that share the same diploid parent

is analogous to self-fertilization in diploid-dominant plants and animals. Further, asexual reproduction can yield the dominance of either haploid or diploid individuals. Using *Mastocarpus* sp. and *Gracilaria vermiculophylla* as examples, I will explore the genetics and evolutionary ecology of range expansions across spatial scales. Asexuality results in the loss of the crustose life history stage in *Mastocarpus* sp. The maintenance of these patterns of geographic parthenogenesis do not appear to be driven by the same mechanisms that underlie these patterns in higher plants or animals. Rather, the loss of a free-living stage likely enables range expansions along tidal and latitudinal gradients. In the case of *G. vermiculophylla*, the ecological shift from attached to unattached thalli, ostensibly necessitated by the invasion of soft-sediment habitats, correlated with shifts from sexual to asexual reproduction during the Northern Hemisphere invasion. A labile mating system likely facilitates colonization success and subsequent range expansion, but the long-term eco-evolutionary impacts will depend on which ploidy stage is lost and the degree to which asexual reproduction is canalized.

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HOLOCENE OCEANOGRAPHIC VARIATION IN THE GODTHÅBSFJORD REGION, SW GREENLAND, USING DIATOM PROXY

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Godthåbsfjord region is composed of a sill fjord (190 km-long) and the adjacent continental shelf located in SW Greenland. The fjord is composed of a number of branches with surface area of 2,013 km² and is directly connected to the Greenland Ice Sheet. The shelf area is influenced by the oceanic current carrying Atlantic water masses. Study material was collected from the shelf area (64° 27.0694"N, 52° 47.5783"W) from 495 m water depth. Sediment core SA13-ST3-16R was retrieved using Rumohr Lot corer during R/V Sanna cruise in 2013. The core was sampled at 2 cm intervals and samples were subsequently processed using standard laboratory procedures and light microscopy. The aim of our study is to investigate the

changes in diatom species composition in Godthåbsfjord region, SW Greenland over the past centuries based on high-resolution diatom record. We will present qualitative and quantitative reconstruction of oceanographic changes, i.e. sea ice concentration and surface water temperature. The quantitative reconstruction will be done using a recently developed diatom calibration dataset for the transfer function – based reconstructions of the West Greenland paleoenvironment.

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

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The EU FP7 project PUFACHain aims to substantiate the industrial development of a sustainable production of omega-3 fatty acids from microalgae, in particular DHA and EPA. Since conventional PUFA sources are becoming more and more limited, microalgae present a promising alternative that provides stable product concentrations and avoids unwanted by-products. Two culture collections, the SAG (Göttingen University) and the CCCryo (Fraunhofer), provided algal strains from temperate and polar regions. The combination of temperate and cold-adapted strains may allow PUFA outdoor production in Middle Europe throughout entire year (Algae Crop Rotation principle). During extensive screening the EPA, DHA and ARA content in dry algal biomass was quantified for more than 100 strains from the SAG culture collection. EPA ranged from 0.04% to 4.6% of dry mass and DHA from 0.03 to 1.8%. The EPA and DHA concentrations varied both among

different species as well as different strains of a single species. A handful of previously unexplored and known EPA/DHA producers, mostly representatives of Eustigmatophyceae (Stramenopiles) and Dinophyta, were selected for further upscaling and extraction experiments following the biorefinery concept. The growth conditions (light, temperature, nutrients concentration) were further optimized for biomass and target product accumulation in the lab-scale (up to 250 ml). The influence of the different cultivation factors on DHA/EPA yield varied from strain to strain but the increase of EPA/DHA yield was observed with favorable conditions for biomass accumulation. The genetic characterization of PUFA producing strains assured reproducibility and accuracy of the PUFA production.

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TEMPERATURE AS A FACTOR DETERMINING BIOGEOGRAPHICAL DISTRIBUTION OF THE DIATOMS (BACILLARIOPHYTA) AND THE ECOSYSTEM PROCESSES OFF THE COAST OF SOUTH AFRICA AS A RESULT OF MODERN CLIMATE CHANGE

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In the general opinion of biologists and oceanographers, temperature is one of the most important factors influencing

the biodiversity of the seas and oceans. This research project examines the impact of changes in seawater temperature on the species composition of benthic and planktonic diatom assemblages along the coasts of South Africa. The diatom composition is influenced by modern climate changes and primarily by the rise in global water temperature. Diatoms are considered to be one of the best bioindicators, and their response to temperature changes is commonly used in environmental reconstructions of Earth's geological past. South African demonstrates unique conditions to observe the effects of global warming and climate change in the ocean's coastal zone, due to the presence of two large ocean current systems: the Agulhas Current flowing southwards along the east coast of South Africa and the Benguela Current flowing northwards along the west coast. These currents differ in terms of oceanographic conditions and productivity. The Benguela Current is associated with upwelling cells and therefore the system is considered as one of the most productive ocean zones on Earth. The occurrence of extremely different conditions (mainly related to water temperature) means the diatom assemblages along the coasts of South Africa bear the mark of ocean currents. Sampling diatoms at selected sections on the South African coast, together with their growth in culture, will allow for the documentation of the diatom assemblage's biodiversity. Our research, supported by long-term in situ temperature measurements, should determine any temperature rise and its effect on the species composition of diatom flora along the South African coast.

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DEFINING VIRAL INFECTION STATES OF A BLOOM-FORMING MARINE ALGA WITH SINGLE-CELL DUAL RNA SEQUENCING

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Host-pathogen interactions are highly dynamic and complex, involving various host responses and pathogen hijacking strategies. Studying host and virus gene expression

patterns by sequencing whole populations (dual RNA-seq) can reveal key genes involved in the infection cycle. However, it tends to mask differences between individual cells, therefore hampering the ability to discern between diverse phenotypes and to discover rare subpopulations. In contrast, single-cell genomics can potentially uncover heterogeneity of host responses and infection outcomes within populations of infected cells. We characterized the variability in host and viral gene expression during the infection through single-cell dual RNA-seq of *Emiliania huxleyi*, a marine bloom-forming unicellular alga. Individual algal cells were sampled from uninfected and infected cultures using fluorescence-activated cell sorting and their transcriptomes were profiled using the massively parallel RNA single-cell sequencing method. Hundreds of unique host or viral transcript molecules were detected in each cell, with the latter being highly variable within the infected population. Based on infection treatments and viral transcript counts, we defined five subgroups of cells. A notable increase in ribosomal RNA transcripts was detected in subgroups from the infected culture. Among the cells with a high number of viral transcripts, two subpopulations were identified that showed differential expression of viral genes. The major capsid protein gene, important for virion assembly, was mainly expressed in a small subpopulation of cells with low levels of other viral genes. The five infection states also had distinct profiles of various host genes, including those of primary metabolism, energy production and lipid metabolism. We demonstrate that single-cell genomics provide a novel approach to reconstruct the timeline of pathogenicity. Future studies with extensive sampling are expected to improve our ability to reveal more detailed infection states and to map them to unique host phenotypes.

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INTER-ANNUAL DIFFERENCES IN PHYTOPLANKTON SPRING BLOOM COMMUNITY STRUCTURE IN A HIGH ARCTIC FJORD (ADVENTFJORDEN, SVALBARD)

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We investigated the size and trophic structure of the annual planktonic protist community structure in the ice-free Adventfjorden in relation to environmental factors. Our high-resolution (weekly to monthly) study was conducted in a period of high inter-annual variability in Atlantic water advection to the fjord (January 2012 – October 2013). The study showed clear inter-annual differences in the spring community structure, most likely the response to various hydrographical conditions. In both years, the spring blooms, manifested by a sudden increase in the total protist abundance and biomass, took place in the second half of April, i.e., about a month earlier compared to the ice-covered West Spitsbergen fjords. Bacillariophyceae dominated the spring bloom when local water masses prevailed, while *Phaeocystis pouchetii* dominated the spring bloom when Atlantic water prevailed. In addition, the study showed that intrusions of Atlantic waters can strongly modify the quantitative composition of local communities in Adventfjorden, as evidenced by, *inter alia*, an order of magnitude greater protist abundance in the spring of 2012, corresponding with a substantially higher phototropic protist biomass (chlorophyll *a*) in surface waters adjacent to Isfjorden. In the light of the progressive increase of Atlantic water inflow to the Arctic, the shift from Bacillariophyceae-dominated to *P. pouchetii*-dominated spring bloom may become more frequent and can negatively affect the energy transfer efficiency in the marine food web.

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PCR IDENTIFICATION OF TOXIC EUGLENID SPECIES EUGLENA SANGUINEA

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Euglena sanguinea is one of the first green euglenid species described in the literature. It is a cosmopolitan species, which can be found in shallow, calm, eutrophic freshwater systems. *Euglena sanguinea* is the only known toxic species of euglenids, which forms blooms causing tangible losses to fish farms. It produces euglenophycin, a toxin similar in structure to solenopsin, an alkaloid from fire ant venom. Recently a specific mass spectrometric method of identification and quantitation of euglenophycin was developed to facilitate monitoring for that toxin in freshwater ponds. Despite recent taxonomic verifications, correct identification of *E. sanguinea* is still difficult, especially for less experienced researchers. Herein we describe the simple method basing on two-rounded nested PCR amplification of nSSU rDNA fragment to identify a single *E. sanguinea* cell. The method will further facilitate monitoring of reservoir water, especially for estimating the risk of toxic blooms.

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NEW FRESHWATER DIATOM GENUS OKHAPKINIA GEN. NOV. FROM LAOS (SOUTHEAST ASIA), WITH NOTES ON MOLECULAR PHYLOGENY OF THE FAMILY SELLAPHORACEAE

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A new monotypic diatom genus from Laos is described, *Okhapkina alexandrii* Glushchenko, Kulikovskiy & Kociolek gen. et sp. nov. The new genus is described on the basis of light and scanning electron microscope observations. This genus is similar morphologically to the genus *Sellaphora* Mereschkowsky on the basis of several shared features. These features include presence of uniseriate striae, internal hymenate pore occlusions, large, elongate helictoglossae, round depressions at the valve face mantle junction, straight

internal raphe slit, presence of longitudinal lines positioned on either side of the axial area, and striae arrangement and shape. The new genus is diagnosed by having an external, undulate raphe slit, absence of polar bars, presence of massive basal siliceous layer, and presence of rims and external thickenings around the areolae, central raphe ends curved to different sides internally and very prominent elongated hymenes internally. In all of these features the new genus differs from *Sellaphora*. The shared features between *Okhapkina* gen. nov. and *Sellaphora* suggest the new genus should be assigned to the Sellaphoraceae Mereschkowsky. Similarities between *Okhapkina* gen. nov. and other newly-described species are discussed. We consider the newly described genus *Altana* Kulikovskiy, Lange-Bertalot & Metzeltin, which shares the feature of external silica outgrowths with *Okhapkina* gen. nov. to be part of the family Cavinulaceae D.G. Mann. The genus *Buryatia* Kulikovskiy, Lange-Bertalot & Metzeltin described from Lake Baikal may represent another genus closely related to *Sellaphora*, and we suggest it is also a member of the family Sellaphoraceae. Molecular phylogeny of some genera and species from the family Sellaphoraceae is discussed.

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MICROPHYTOBENTHIC FLORA IN A SHALLOW LAGOON OF THE SOUTHERN BALTIC SEA

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The Darß-Zingst Bodden Chain is a shallow lagoon of the southern Baltic Sea in Germany. This lagoon is characterized by a high phytoplankton biomass, which forms a huge photosynthetic benthic boundary layer on top of the sediment. Therefore, a true microphytobenthos community was not expected. However, some typical large benthic diatoms occurred in net (>55 µm) plankton samples. We focused on the microphytobenthic flora, especially benthic diatoms. Each sediment sample from Zingst was bleached with hydrogen peroxide to remove organic matter. Quantitative analyses were carried out, with 300 valves counted

in each cleaned sample for species composition and relative abundance. Furthermore, cleaned samples and the surface of sand grains were observed using electron microscopes (SEM & TEM). This was done also with epiphytobenthos from important submersed macrophytes (Charophyta).

A total of 72 species in 34 genera were recorded from all samples (sediments and algae). Abundant diatom species in sediment samples were: *Pseudostaurosira brevistriata* (Grunow) D.M.Williams & Round, *Pseudostaurosiropsis geocollegarum* (Witkowski) E.A.Morales and *Staurosira construens* var. *venter* (Ehrenberg) P.B.Hamilton. The common morphological features of these species are araphid and linking spines, which join frustules together in a ribbon-like colony. Compared to the attached diatoms on *Chara baltica*, the benthic diatoms occurring in sediments were relatively small and it was difficult to discern their specific features using a light microscope. Therefore, particular attention was paid to small diatoms which have been probably overlooked before. The most abundant diatom species on *Chara baltica* was *Rhoicosphenia abbreviata* (C.Agardh) Lange-Bertalot and its relative abundance was over 70%.

To reveal differences in species composition and dominance of diatom taxa, more samples will be seasonally collected from various sites at different salinities along the lagoon and investigated further.

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REGISTRATION OF NAMES AND TYPES OF ALGAE BY PHYCOBANK

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Phycobank is to become the registration system for nomenclatural novelties and changes in phycological research, such as new names, new combinations, new lectotypes, or epitypes (Barkworth et al. 2016, *Taxon* 65: 672). Registration will check conformity with the rules of the International Code of Nomenclature for algae, fungi and plants (ICN) but will be taxonomically neutral. Phycobank will provide a central registration service for such nomenclatural acts,

which can be submitted either directly by nomenclatural authors or automatically via cooperating publishers. Each new name gets a globally unique http-based Phycobank Identifier that can be included in publications (e.g. Jahn et al. 2017, *PhytoKeys*). This HTTP URL references the corresponding data and metadata in Phycobank in human- and machine-readable formats. Additionally, Phycobank can be searched by name, registration date, category of nomenclatural novelty, and type specimen. Feedback mechanisms will be incorporated for community involvement. Appropriate treatment of ambireginal taxa will also be considered. Interoperability with the global names infrastructure will be achieved via standardized web services.

Upon registration, Phycobank will feed evaluated name data into the Global Names Architecture via the Catalogue of Life. A close co-operation with DiatomBase (diatoms), WoRMS (marine taxa), IPNI (author names) and Index Nominum Algarum (limnic and marine algae), and possibly AlgaeBase is envisaged. In addition, Phycobank will also cover all specialties needed by the phycological community: treatment of ambireginal taxa (e.g. bluegreen algae, dinoflagellates, euglenoids), algal group-specific provisions, later starting points, lecto- and epitypes, and figures of algae which cannot be preserved.

The public trial run of Phycobank started in 2017 with data entered by Phycobank staff. A web interface allowing data entry by nomenclatural authors is under construction. The automatic submission workflow (for publishers) is in the planning phase.

Phycobank is supported by a DFG Grant (JA 874/8-1) and available at <http://phycobank.org>.

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OSTREOPSIS OVATA FUKUYO 1981 ALONG THE EASTERN ADRIATIC COAST

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Ostreopsis spp. are benthic dinoflagellates that are known to cause harmful algae blooms. They are known producers of a range of palytoxins and ovatoxins. During bloom events

those toxins can be found in high concentrations in benthic biofilms, in the water column and in coastal aerosols. *Ostreopsis* spp. distribution and related harmful algae blooms are well documented along the western Adriatic coast and parts of the western Mediterranean. Little was known about its distribution along the eastern Adriatic coast which presents very different ecological conditions to the western Adriatic coast. A high resolution sampling regime along the eastern coast of the northern Adriatic revealed a dense population structure and high abundances along that coastline. Samples from the middle and southern Adriatic confirmed the presence of *Ostreopsis ovata* along the entire eastern Adriatic coast. Our results demonstrate that oligotrophic rocky shores can be a very suitable habitat for *O. ovata*. Massive bloom events with record abundances and toxin concentrations are reported. The population along the north-eastern Adriatic coast share one ITS1-5.8S- ITS2 genotype with most of the populations recorded around the Mediterranean.

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INTERSPECIFIC VARIATION IN METABOLIC RESPONSE TO P LIMITATION HELPS EXPLAINING SYMPATRIC CONGENERIC SPECIES DIVERSITY (IN THE NORTHERN ADRIATIC)

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The northern Adriatic is characterized by a multitude of steep and quickly changing gradients (e.g. nutrients), both temporal and spatial. The current systems and the major

freshwater input (River Po) in particular generate strong gradients in nutrient concentrations with an expressed N/P imbalance and sustained phosphate limitation. This results in a highly structured planktonic ecosystem that allows for a high level of sympatric congeneric species diversity. We analyzed several such sympatric congeneric species with respect to their metabolic reaction towards phosphate limitation. Here we report data on species-specific growth rates under different nutrient regimes, phosphate uptake rates, alkaline phosphatase activity, localization and activation patterns and characteristics of alkaline phosphatase activity. Our results demonstrate a high interspecific variation in metabolic responses to phosphate limitation in sympatric congeneric species. The ecological significance and function appears therefore to be defined on the species level and appears far from homogeneous within genera.

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EPIGENETIC REGULATION IS INVOLVED IN THE FORMATION AND DIFFERENTIATION OF PROTOPLASTS IN THE MARINE GREEN ALGA BRYOPSIS PLUMOSA

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When the cell membrane of the marine coenocytic green alga *Bryopsis plumosa* is destroyed, the protoplasm expelled into the seawater generates numerous protoplasts in vitro. Protoplasts were surrounded by a polysaccharide envelope from the beginning of formation, which acted in a similar way to the cell membrane until this developed within several hours. We constructed 2-DE protein maps and a transcriptome database of protoplasts at different stages of formation and attempted to elucidate characteristic genes expressed at each stage. The profiles of the proteome as well as the transcriptome changed dramatically at each stage of the cell rebuilding process. Most proteins which were up-regulated during the early stage of cell rebuilding disappeared or reduced significantly by 24–48 h. About 70–80% of protein spots detected 48 hours after the wounding were newly appeared ones, which has rarely been seen in any other proteomes studied to date. DNA methylation seems to be involved in the cell-rebuilding process of *B. plumosa*. DNA methylation is often utilized to silence and regulate genes without changing the original DNA sequence. To prove this hypothesis, DNA methylation assay was carried

out using regenerating protoplasts at different stages. A dramatic de-methylation occurred in the regenerating cells for 24 hours after the release of cytoplasm in seawater. These results show that an epigenetic modification drives differential expression of the large gene sets specifically regulated at each developmental stage of the cell-rebuilding process in *B. plumosa*.

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UNCOVERING THE GENETIC BASIS OF BACTERIA-INDUCED MORPHOGENESIS IN *ULVA MUTABILIS*

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The green marine macroalga *Ulva mutabilis* (Chlorophyta) requires the presence of specific bacteria, or the morphogenic compounds they produce, to complete normal development. Although the identification of the molecules that mediate this mutualism steadily progresses, the genetic control that underlies it still needs to be elucidated. To investigate which *Ulva* genes are involved in the regulation of bacteria-dependent morphogenesis we created insertional mutants based on the integration of a phleomycin resistance marker in the *Ulva* genome. After genetic transformation, *Ulva* individuals that survived phleomycin selection and potentially showed aberrant mutant phenotypes were recovered. Next, we tested different molecular methods to identify genomic insertion sites and determined that adapter-ligation PCR was the most efficient way to retrieve these regions. Mutants were identified that carry insertions in intergenic – repetitive – sequences, but, interestingly, also in genes encoding a receptor-like protein and a WD40-repeat-containing protein. Subsequent in silico analysis showed that these genes are conserved in other chlorophyte algae, but did not shed light on their physiological role in *Ulva*. Currently, we are further studying the extent to which gene function in these mutants is disrupted and how it affects the *Ulva* phenotype.

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MARINE MICROALGAE AS SOURCES OF PHOSPHOLIPIDS AND STEROLS FOR USE AS NUTRACEUTICALS AND ENCAPSULATION SYSTEMS

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Addition of nutraceuticals to food products shows promise as diet-based prevention for many chronic diseases. Nutraceuticals are often hydrophobic and not easily incorporated into food products. Phospholipids from microalgae are ideal for the production of liposomes, allowing incorporation of the hydrophobic compound of interest, and potentially providing omega-3. Phospholipids have the ability to increase adsorption of DHA in infants, and reduce cholesterol and hepatic fibrosis in mammalian models. Three species were studied, (*Cylindrotheca fusiformis* CCAP 1017/2, *Nannochloropsis oceanica* CCAP 849/10 and *Isochrysis galbana* CCAP 927/1). Lipid and pigments were extracted from samples taken throughout the growth phase. Percentage total lipid extract was determined and lipid classes were analysed by HPTLC and scanning densitometry. *C. fusiformis* phospholipids varied over the growth of the culture (3.11-14.51% of TL). *N. oceanica* had an overall high proportion of phospholipids, highest at day 5 (21.37% of total lipid), corresponding to the beginning of logarithmic growth, with the proportion of phospholipids remaining high throughout. Detectable phospholipid in *I. galbana* was only present at days 10 and 13, (1.03-2.79% of TL). Photoautotrophic microalgae, produced without carbon substrates and grown on non-arable land or using water sources unsuitable for terrestrial plants, confer advantages over terrestrial plants and bacteria. Microalgae have potential as future sources of complex polar lipids and phytosterols and for use as additives or delivery systems for high value nutraceutical products.

USING HIGH THROUGHPUT SEQUENCING TO INVESTIGATE DIVERGENCE IN NON MODEL ORGANISMS: AN EXEMPLE IN THE DINOFLAGELLATE ALEXANDRIUM MINUTUM

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Understanding speciation in the highly dispersive and seemingly homogeneous pelagic environment for micro-organisms living as free drifters in the water column remains a challenge. Are the divergence events associated with ecological niche differentiation, or are they the result of neutral divergence? What are the cellular functions associated with these events? Reverse ecology approaches (i.e. using genomic data as a starting point to investigate ecology, without any a priori on the phenotypes of interest) may be a good starting point to better understand the forces at play during micro- eukaryotes speciation events. We identified an incipient speciation event within the toxic dinoflagellate *Alexandrium minutum*. Using more than 400,000 SNPs, we showed: 1. that the observed divergence may be the result of an ancestral divergence in allopatry followed by a secondary contact resulting in some gene flow, but heterogeneous across the genome; 2. A strong overrepresentation of non-synonymous polymorphism, suggesting the importance of selective pressures as drivers of the divergence; 3. That the most divergent genes are homologs to genes involved in calcium/potassium fluxes across the membrane, calcium transduction signal and saxitoxin production, giving some insights on the functional bases of the divergence.

PROTEOMIC ANALYSIS OF PYROPIA YEZOENSIS MUTANT INDUCED BY GAMMA IRRADIATION

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The present study describes isolation of an elevated-temperature-tolerant mutant of *Pyropia yezoensis* (Py500G) created by g-irradiation. The growth rate of the Py500G mutant strain is greater than that of the wild type (WT) at 20°C. We conducted a comparative proteomic analysis to identify novel proteins involved in elevated-temperature tolerance

in the marine alga *P. yezoensis*, in strains WT and Py500G (mutant). Protein extracts for each temperature condition (12°C, 18°C, and 20°C) were separated by 2-dimensional gel electrophoresis and changes in the proteome of the fronds were analyzed. Approximately 218 protein species were detected, 12 of which were differentially expressed. The mutant Py500G strain showed six over-expressed, three under-expressed and three equally-expressed proteins as compared to the WT strain grown at 20°C. These findings indicated that the growth rate of Py500G was significantly higher and that this strain resists elevated-temperature stress by changing expression of certain proteins, six of which (upregulated) are antioxidant enzymes potentially involved in photosynthesis and protein synthesis. This result may be useful for understanding the response of *Pyropia* against heat stress and the strain will be potentially applicable in nori cultivation.

NEW AUXILIARY CELL AMPULLAE BASED ON FOLIOSE RED ALGAE, NESOIA GEN. NOV. (HALIMENIACEAE, RHODOPHYTA) FROM KOREA

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The family Halymeniaceae is characterized by unique reproductive organs composed of carpogonial branch ampullae and auxiliary cell ampullae. The type of auxiliary cell ampulla has been considered as the key characteristic in the family Halymeniaceae. The continuous surveys into subtidal region from Korea including morphology and *rbcL* molecular analyses give a chance to reveal a new evolutionary lineage in the generic level. As a result, we propose a new genus, *Nesoia* gen. nov., with description of *N. delicatula* sp. nov., *N. pulchella* sp. nov., and *N. latifolia* comb. nov. which is a new combination from *Halymenia latifolia*. The genus *Nesoia* gen. nov. shows a foliose vegetative thallus composed of single layered cortex and anticlinal medulla. This new genus is characterized by monoecious gametophytes and auxiliary cell ampullae consisting of ampullary filaments branched to three orders. The auxiliary cell is the basal cell of second-order branches, that are borne on the secondary or third cell of first-order ampullary filaments and bear two lateral filaments. This new type of auxiliary cell ampulla is intermediate between *Halymenia*-type and *Aeodes*-type, and will provide more effective resolution to

delimit taxonomic boundaries among genera of Halymeniaceae.

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SPECIES BOUNDARY OF SCINAIA JOHNSTONIAE (NEMALIALES, RHODOPHYTA) FROM THE NORTHERN PACIFIC USING MOLECULAR ANALYSIS

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The genus *Scinaia* has been identified to species level based on the morphological characters, such as pattern of thallus segments, size and shape of utricles, shape and layer of pigmented hypodermal cells, cortical arrangement in surface view, and location of spermatangial sori. *Scinaia articulata* from California and *S. tokidae* from Korea and Japan were synonymized as *S. johnstoniae* on the basis of morphology only. To reveal the species boundary and distribution of *S. johnstoniae*, we conducted *rbcL* analysis for the specimens of cylindrical type *Scinaia* from Korea and California, USA. We confirmed three genetic groups of *S. johnstoniae* specimens from the type locality, California including specimens from Korea. One Korean group revealed as *S. tokidae*, distinguished from *S. johnstoniae* by utricle size, the other groups composed of *S. articulata* and *S. johnstoniae*. *S. johnstoniae* exhibited 5.0% and 7.7% interspecific divergence with *S. articulata* and *S. tokidae*, respectively. The *rbcL* analysis of *Scinaia* resolved the limitation of species identification based on the morphology only. In conclusion, two synonyms of *S. johnstoniae* should be resurrected as taxonomic entities, *S. articulata* and *S. tokidae*.

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ASSESSING PHYLOGENETIC SIGNALS AND HOMOPLASY IN MORPHOMETRIC DATA OF PSEUDOPEDIASTRUM BORYANUM AND MORPHOLOGICALLY SIMILAR TAXA

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Morphological variability of *Pseudopediastrum boryanum* (Chlorophyceae, Sphaeropleales, Hydrodictyaceae), a microscopic green alga forming coenobia with two processes on each marginal cell and granulate wall ornamentation, can be described using a number of characteristics. The most prominent are cell number, marginal cell shape and size, as well as granule density. In order to test whether any of them match the phylogeny of *Ps. boryanum* and its similar taxa, 13 monoclonal strains were chosen. They represent some *Ps. boryanum* varieties, including *boryanum* (2), *cornutum* (2), *forcipatum* (1), cf. *forcipatum* (1), *longicorne* (2), and *Ps. alternans* (1), *Ps. cf. alternans* (1), *Ps. integrum* (2) and *Pediastrum berolinense* (1). Each strain has specific morphometric parameters. Mapping the information on cell number, marginal cell shape and size, and ornamentation type onto the phylogeny does not show significant phylogenetic signals in any characteristic. The same results are obtained regardless of whether analyzing the strains separately or analyzing them as four clades (*P. berolinense*, *Ps. boryanum*, *Ps. integrum* and *Ps. cf. alternans*). This indicates that the morphological variability of all four characteristics is strongly connected with homoplasy at various taxonomic levels, both between *Ps. boryanum* and morphologically similar species, as well as between *Ps. boryanum* varieties.

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MORPHOLOGICAL CHARACTERIZATION OF CYANOPROKARYOTES FROM SANTA CLARA HYPERSALINE COASTAL POND, YUCATÁN, MÉXICO

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Coastal habitats in the Yucatan peninsula have a Karstic origin and are predominantly coastal lagoons frequently modified for salt production. This human activity generates a hypersaline biotope that is colonized by special microbial communities. Hypersaline ponds have seldom been studied in the Yucatan peninsula, México, therefore their

cyanoprokaryote diversity is almost unknown. The aim of this study was to obtain a comprehensive description of the photosynthetic microbial diversity of the Santa Clara hypersaline biofilms. For this study biofilms and crusts from a Santa Clara hypersaline pond were collected during spring of 2017, samples were preserved both dry and in 4% formalin in marine water.

Several culture strains are in the process of isolation. Environmental conditions were: temperature 37.7°C, salinity 10‰ and pH 8.1. Samples, whether dry or submerged, were examined with BF, DIC in an Olympus BX 51, micrographies from confocal microscope were also obtained in order to get a more complete morphological characterization. The taxonomic diversity found in this biotope was reasonably high as it is represented by more than 20 morphotypes belonging to 12 families and 4 orders of Cyanoprokaryota. The most common were: *Aphanothece* sp., *Chroococciopsis* sp., *Halospirulina* cf. *tapeticola* and several morphotypes of *Phormidium*. Most populations could only be identified to genus or cf. level as they showed thallus, cellular dimensions or ecological distribution different from the most related species. Morphological descriptions as well as micrographies are provided for all morphotypes. As a result a *Pleurocapsa* morphotype, *Phormidium* cf. *lucidum* and *P. acuminatum* are reported for the first time for Yucatan hypersaline biotopes.

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CRYOPHILIC ALGAE SURVIVE IN SPACE

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Within the multinational project BIOMEX two strains from the CCCryo collection were tested for survival under space- and mars-like conditions on board the EXPOSE-R2 space platform on the outside of the International Space Station (ISS) between 2014 and 2016. The two strains

originated from cold habitats on Spitsbergen (Norway) and Antarctica, and as extremophiles with their adaptation to cold and desiccation stress they appeared to be good candidates that might survive the harsh conditions in space. Strain CCCryo 101-99 of cf. *Coenochloris signiensis* (Chlorophyceae, Chlorophyta) is a green microalga from Spitsbergen capable of producing carotenoid-rich resting stages. Strain CCCryo 231-06 of *Nostoc* sp. (Cyanophyceae, Cyanobacteria) is a cyanobacterium from Victoria Land in Antarctica, capable of forming filaments embedded in a gelatinous colony and also akinetes. After numerous simulation tests on Earth, samples were prepared as desiccated resting stages/colonies on different solidified culture media and mars-and lunar- regoliths, placed on the outside of the ISS and exposed to the following parameters for 15 months: temperature changes between -20 and +47°C, low pressure between space vacuum and a mars-like atmosphere of 1,000 Pa with >95% CO₂, and UV-A, -B and -C radiation of 439 MJ m⁻² (l = 200–400 nm) as well as cosmic radiation of approx. 500 mGy. Preliminary tests had shown that UV- radiation had the most fatal impact and thus, 0.1% quartz/MgF₂ filters were used to reduce UV-radiation to 0.4 MJ m⁻² on the ISS. On their return from space the samples were inoculated in fresh culture medium. Within 12–28 days almost all samples developed actively growing populations. The green alga produced zooids, young and adult vegetative cells and in nutrient-depleted cultures also carotenoid-producing cysts. *Nostoc* showed young filaments indicating recovering growth. Agar slants were inoculated from all “space”-samples and show growth comparable to laboratory controls.

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BENEFITS OF THE ALGAE CROP ROTATION (ACR) PRINCIPLE IN ALGAL MASS PRODUCTION

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Producers of microalgal mass often face the problem that their outdoor photobioreactor systems become uneconomic for a specific period of the year, because growth rate and metabolite production of the algal strain decrease when environmental parameters are unfavourable. Temperature and light are the most decisive factors in this regard. Within the framework of the FP7 EU-project *PU-FAChain*, numerous strains from the SAG and CCCryo algal collections were screened for the best DHA and EPA producers. Two EPA-producing algal strains, one mesophilic and the other cryophilic, were compared with regard to their EPA production in relation to temperature and light. We show that each of the strains has very different temperature and light preferences. EPA yields in the mesophilic strain are best when grown at 20–30°C and at PAR photon flux densities of 30–1,000 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$, whilst the cryophilic strain prefers temperatures between 4–20°C and 5–100 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$, respectively. It is obvious that at locations in Middle or Northern Europe where there are large differences in temperature and light between summer and winter, one of the strains has its optimum conditions during the warmer and the other during the colder seasons. Alternating two such strains in the course of a year according to the prevailing environmental conditions, i.e. applying our proposed Algal Crop Rotation (ACR) principle, can result in an annual overall increase in productivity of an algal mass production plant without investing extra energy and costs in major external temperature or light regulation. We modelled conditions at four European locations with strong differences in their climate, using laboratory and pilot plant results, and found that the annual overall EPA-yield could be increased by 50% when using a cryophilic strain during the colder and darker seasons in Middle Europe (Hamburg) and by up to 150% when producing in Northern Europe (Oslo, Trondheim).

USE OF MOLECULAR METHODS IN RESEARCH ON MARINE BENTHIC DIATOMS AND ITS BENEFITS FOR BIODIVERSITY AND ENVIRONMENTAL RECONSTRUCTIONS OF THE COASTAL ZONE OF THE BALTIC SEA AND BEYOND

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Between 2013 and 2015, we sampled benthic diatoms from the coasts of the western Pacific Ocean, Indian Ocean, eastern Atlantic Ocean and the Mediterranean Sea as well as from the Baltic Sea, particularly from the Gulf of Gdańsk, Pomeranian Bay and Danish Straits. Particular attention was paid to small araphid forms, which constitute an important component of diatom assemblages in the marine littoral for their contribution to biodiversity and potential for bioindication and environmental reconstructions in economically-important coastal lakes, estuaries, lagoons and fjords of the Baltic coasts. These diatom taxa (*Fragilaria*, *Opephora*, *Staurosira* or *Pseudostaurosira*) were traditionally classified very broadly by simple morphological characters, but due to this simplicity and their small size (<20 μm) identification can be difficult. As a result, even when conducted by an expert in diatom taxonomy, the true genetic diversity present can be under-represented. To better understand this diversity, we have characterized both the morphology (by light and electron microscopy) and DNA sequence data (nuclear-encoded small subunit ribosomal RNA and chloroplast encoded *rbcL* and *psbC*) from dozens of strains of small araphid diatoms isolated, cultured and maintained in the University of Szczecin culture collection (SZCZ) with the goal of creating a complete morphological and molecular database of small, marine littoral diatoms for use in bioindication studies. Based on these observations, we used the molecular phylogeny to drive our proposal of useful characters to distinguish the genera and species of the ‘staurosiroid’ diatoms, to better

represent their diversity and evolutionary history, including proposing several new genera. For example, Baltic Sea strains identified as *Opephora* belong to two new genera, and the widely-recognized Baltic Sea diatom *Fragilaria subsalina* (= *F. virescens* var. *subsalina*) also belongs in a new genus, along with other “staurosiroid” strains which have been used in past.

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EFFECT OF NITRATE: PHOSPHATE ON GROWTH AND MICROCYSTIN-LR PRODUCTION OF MICROCYSTIS AERUGINOSA

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Microcystin-LR (MC-LR), a problematic potent cyanotoxin, is produced by a variety of cyanobacteria. The presence of MC-LR in lake water is a severe threat to human health as well as an environmental concern. *M. aeruginosa* was isolated from a pond in Shantou University, China. Changes in the Microcystin-LR content of *Microcystis aeruginosa* were investigated in five different N:P concentrations [nitrate (μM) 117.7, 175, 235, 282.4, 329.5 and phosphate (μM) 110, 230, 460, 750, 980 and N:P(μM) 117.7–110, 175–230, 235–460, 282.4–750, 329.5–980 μM]. Cultures were acclimated to the respective temperature (18, 25, 30°C) and light (850, 1,600, 2,300 lux) regimes for 28 days and maintained in BG-11 medium. Nine samples have been studied based on the highest growth stages. *M. aeruginosa* was phylogenetically identified by the 23S rDNA plastid marker and a fragment of *mcyA* gene encoding for microcystin synthetase was detected, indicating its potential for microcystin production. High performance liquid chromatography analysis confirmed *M. aeruginosa* produces microcystin-LR. The results showed that a P-fixed concentration of 460 μM (25°C, 30°C) had an obvious influence on the growth of *M. aeruginosa* and the highest content of

microcystin-LR (1,447 $\mu\text{g g}^{-1}$; 1,306 $\mu\text{g g}^{-1}$), followed by N:P ratio of 235–460 μM (18°C) where we recorded 1,228 $\mu\text{g g}^{-1}$. There was a highly significant relationship between the microcystin-LR content of *M. aeruginosa* and the chlorophyll-*a* concentration in the culture during the incubation ($p < 0.0001$). Microcystin-LR content was higher at 25°C with P-limited conditions.

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RESTING CYSTS OF POTENTIALLY HARMFUL DINOFLAGELLATES IN KOREAN COASTAL AREA

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The frequency of harmful algal blooms (HABs), caused by the cyst-forming dinoflagellates, has been increasing dramatically since the 1980's in Korean coastal waters. Although the distribution of dinoflagellate resting cysts has been widely investigated in Korean coastal areas, morphological characteristics of the resting cysts of potentially harmful dinoflagellates are still unclear. Here, we provide detailed descriptions of these potentially harmful dinoflagellate cysts to increase understanding of the morphological characteristics and biogeographic distribution of potentially harmful dinoflagellates. Sediment samples were collected from 51 stations in the southern coastal area, Korea. Viable resting cysts were isolated and induced to excysts. Identification was based on morphological characteristics and molecular phylogenetic positions of the germinated cells. Nine potentially harmful dinoflagellate species were identified: seven potentially toxic species and two potentially bloom-forming species. The resting cysts of *Gymnodinium aureolum*, which has a smooth, thin wall and many pale lipid globules, were first observed from natural sediments. The presence of resting cysts of potentially harmful dinoflagellates suggests that further research attention and efforts in HAB monitoring and management are required.

TRANSCRIPTOMIC AND MICRORNAOMIC PROFILING REVEALS MULTI-FACETED MECHANISMS TO COPE WITH PHOSPHATE STRESS IN A DINOFLAGELLATE

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While gene regulation can occur at both transcriptional and epigenetic (microRNA) levels, combined transcriptomic and microRNAomic responses to environmental stress are still largely unexplored for marine plankton. Here, we conducted transcriptome and microRNAome sequencing for *Prorocentrum donghaiense* to understand the molecular mechanisms by which this dinoflagellate copes with phosphorus (P)-deficiency. Under P-depleted conditions, G1/S specific cyclin gene was markedly down-regulated, consistent with growth inhibition, and genes related to DOP hydrolysis, carbon fixation, nitrate assimilation, glycolysis, and cellular motility were up-regulated. The elevated expression of ATP-generating genes (e.g. rhodopsin) and ATP-consuming genes suggests some metabolic reconfiguration towards accelerated ATP recycling under P-deficiency. MicroRNAome sequencing revealed 17 microRNAs, potentially regulating 3,268 protein-coding genes. Functional enrichment analysis of these microRNA-targeted genes predicted decreases in sulfatide (sulfolipid) catabolism under P-deficiency. Strikingly, we detected a significant increase in sulfolipid/sulfatide content (but not in sulphoquinovosyldiacylglycerol content) and its biosynthesis gene expression, indicating a different sulfolipid-substituting-phospholipid mechanism in this dinoflagellate than other phytoplankters studied previously. Taken together, our integrative transcriptomic and microRNAomic analyses show that enhanced DOP utilization, accelerated ATP cycling and repressed sulfolipid degradation constitute a comprehensive strategy to cope with P-deficiency in a model dinoflagellate.

SHAPING AN EMBRYO: THE ROLE OF CELL WALLS IN BROWN ALGAL DEVELOPMENT

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The algal cell wall encapsulates every cell. It plays a key role in regulating algal developmental processes by maintaining cell shape, withstanding turgor pressure and modulating its structure to allow growth. We have investigated the role of cell wall mechanics and biochemistry in *Fucus* embryogenesis by characterising normal growth and analysing the outcomes of cell wall alteration. During initial embryo rhizoid tip growth, cell wall stiffness increased from the rhizoid tip towards the thallus. Altering cell wall deposition via the cytoskeleton reduced rhizoid growth and caused an increased cell wall stiffness, correlating with the slower growth observed. A similar pattern was observed when altering the cell wall composition by enzymatic treatment. These data suggest that the mechanics of the cell wall play a role in controlling rhizoid growth by modulating its stiffness in *Fucus* embryos, accompanied by changes in the cell wall composition. We next tested whether alginate biochemistry correlated with cell wall rigidity and growth rate; immunolocalization results were interesting, the soft and fast growing rhizoid appeared to have lower amounts of alginate. We further investigated other cell wall biochemical markers. The advancement of modern techniques and development of new tools have allowed a revised interest in brown algal cell wall research, which further contributes to the knowledge about the universality in its role and function across all cell wall-containing evolutionary groups.

NITROGEN SUPPLY MECHANISMS IN TOXIC DINOFLAGELLATE ALEXANDRIUM OSTENFELDII – THE KEY TO BLOOM EXPANSION IN COASTAL BALTIC WATERS?

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The toxic dinoflagellate *Alexandrium ostenfeldii* forms dense blooms in shallow waters of the coastal Baltic Sea proper during late summer, when concentrations of inorganic nitrogen (N) are low and dissolved organic N (DON) high. If *A. ostenfeldii* is mixotrophic, a trait common among the genus *Alexandrium*, vegetation or phytoplankton derived DON could drive these blooms. We used ¹⁵N-tracer experiments to confirm mixotrophic behaviour of *A. ostenfeldii* through uptake and preference for several N species. Uptake of DON from lysed co-occurring phytoplankton cells was also shown. We performed in situ incubations during dense blooms (6×10^6 cells L⁻¹) in the Åland archipelago, Finland, during August of 2014 and 2016. Nitrogen uptake rate and preference for ¹⁵N-nitrate (¹⁵NO₃), ¹⁵N-ammonium (¹⁵NH₄), ¹⁵N-urea and ¹⁵N-amino acids (¹⁵N-Aa) were measured, with the antibiotic chloramphenicol (CAP) added as a bacterial control. Uptake rates of ¹⁵NH₄ and ¹⁵N-Aa were significantly higher than that of ¹⁵N-urea and ¹⁵NO₃, regardless if bacteria were inhibited. *A. ostenfeldii* consistently preferred amino acids over other tested N species. We visualized the direct uptake of DON from a co-occurring cryptophyte *Rhinomonas nottbeckii*, pre-cultivated on ¹⁵NO₃ and lysed by the released allelochemicals of *A. ostenfeldii*, using second iron mass spectrometry (SIMS). Our findings support the concept that dense blooms of *A. ostenfeldii* utilize DON as N source and that its allelopathic activity facilitates N uptake. Using observational data, a species distribution model was constructed using the Maxent package to examine a possible relationship between *A. ostenfeldii* occurrences and DON-rich coastal habitats. The model showed that distance to shore, temperature, and depth are important explanatory factors. When combined with ratios between background concentrations of inorganic and DON it can improve the prediction of the potential bloom sites of *A. ostenfeldii*. In conclusion we show that nitrogen supply and utilization mechanisms are an important part of understanding bloom expansion of *A. ostenfeldii*.

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ORIGIN AND EVOLUTION OF THE SEXES IN THE BROWN ALGAE

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Although sexual reproduction (meiosis and syngamy) is highly conserved across eukaryotes, the mechanisms that determine whether an individual is male or female are remarkably labile. In genetically controlled sexual systems, gender is determined by sex chromosomes, which have emerged independently and repeatedly during evolution. We are using comparative genomics and evolutionary genetic approaches on the brown algae to gain insights into the origin and evolution of haploid (UV) sex chromosomes. We describe and compare the sex-linked gene content among six brown algal species, belonging to two lineages, the kelps and the Ectocarpales, focusing on gene movement in/out of the U and V-specific regions. We analysed the consequences of sex linkage for the evolution of genes that have been residing in the sex-specific region in the different lineages at different evolutionary times. Our results highlight the dynamic nature of the U and V gene content across the different species compared with autosomes. We will discuss how U and V gene content evolution may be associated with the specific life history traits of each lineage, focusing on the level of sexual dimorphism, life cycle and sexual system.

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RESOLVING DISTRIBUTION PATTERNS OF THREE “CRYPTIC” COLD-WATER DINOFLAGELLATES DOMINATING THE BALTIC SEA SPRING BLOOM

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Commonly occurring spring blooms of dinoflagellates in the Baltic Sea can be dominated by several single-celled species: *Gymnodinium corollarium*, *Biecheleria baltica*, and *Apocalathium malmogiense* (formerly known as *Scrippsiella hangoei*). These species are indistinguishable by routine light microscopy techniques and thus, not detected in Baltic Sea monitoring programs. The three dinoflagellates have different ecological functions due to species-specific nutrient uptake and encystment strategies. Their contribution

to the phytoplankton biomass may vary substantially and affect biogeochemical cycles in the Baltic Sea regarding e.g. sinking of organic matter after the spring bloom. The aim of this study was to identify the three dinoflagellates reliably to species level with the aid of alternative methods and unravel their relative contributions in different parts of the Baltic Sea. Two different methods were used for this purpose: Epifluorescence microscopy of Calcofluor White MR2 stained samples to visualize diagnostic thecal structures and a species detection method based on quantitative polymerase chain reaction (qPCR). Surface water samples ($z = 3$ m) from the Gulf of Finland (GoF) and from the Bothnian Bay to South of Gotland were collected on board R/V Aranda during three subsequent spring blooms (2013, 2014, and 2015; 99 stations) and analyzed accordingly. A comparison of the methods revealed a significant positive correlation between the Calcofluor counts and the qPCR detection. Our results show that *G. corollarium* is the dominant species at most of the sampled stations, *B. baltica* dominance was restricted to the GoF, and *A. malmogiense* generally contributed a negligible fraction of the dinoflagellate biomass in all of the investigated basins. Results of multivariate analysis of qPCR results and environmental data will be discussed to explain these species-specific distribution patterns. Our study highlights the importance of species resolution of the dinoflagellate spring bloom community in the Baltic Sea and the use of complementary identification methods in the Helsinki Commission (HELCOM) phytoplankton monitoring.

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PHYTOPLANKTON COMMUNITY RESPONDING TO A CHANGING ENVIRONMENT – CASE STUDY: SOUTHERN ADRIATIC

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The southern Adriatic is the transitional area where complex water exchange between the Mediterranean Sea and the Adriatic Sea takes place, with pronounced decadal variability of physical and biogeochemical properties. The area is oligotrophic, with low terrestrial nutrient inputs, and is characterized by complex physical dynamics leading to deep-water convection during winter, which in turn enhances thermohaline circulation and advection of waters through the Otranto Strait. The abundances of micro-, nano- and picophytoplankton, community structure, chlorophyll *a* (Chl *a*) concentrations and physical and chemical properties were investigated in the winters of 2015 and 2016 in the area stretching from coastal to open waters of the southern Adriatic. The consecutive winters substantially differed. 2015 was characterized by deep vertical mixing with Chl *a* signal and viable phytoplankton cells detected up to 500 m depth. 2016 exhibited much weaker vertical mixing, detecting Chl *a* only up to 200 m depth. At the same time Chl *a* concentrations were up to four times higher than during the previous winter. Microphytoplankton abundance and composition in both sampling campaigns were an order of magnitude lower than pico- and nanoplankton, and the community was composed mostly of diatoms. Nanophytoplankton was mostly composed of coccolithophores (on average 50%), showing changes in abundance between the years. Picophytoplankton on the other hand, showed community changes between the investigated years, in 2016 *Prochlorococcus* became dominant over *Synechococcus* which had previously been confirmed as dominant in the investigated area.

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THOLOPHORA, N. GEN., A NEW GENUS FOR THE CONOPEATE NITZSCHIA SPECIES

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A study was made of the ultrastructural variation in a group of marine conopeate *Nitzschia* species in Guam that were indistinguishable in LM. The results, together with gene sequences for some species, showed that the group needed to be separated from *Nitzschia* as a new genus, which we call *Tholophora*, conopeum-bearing. Nuclear-encoded ribosomal SSU and chloroplast-encoded *rbcL* and *psbC* sequences from nine strains of *Tholophora* formed a strongly-supported clade sister to a clade consisting of *Achmanthes*,

Craspedostauros, *Staurotropis* and the rest of the Bacillariales, except for *Bacillaria*. All but one of the fourteen taxa are undescribed, the exception being *N. volvendirostrata*, which will be transferred to *Tholophora*. In addition, specimens that initially were identified as *N. spathulata* did not match ultrastructural images in Mann's dissertation and will be described as a new species, *T. spathulatoides*. The names of seven species are after the seven sisters of the Pleiades. The valves in all these species have a central raphe-sternum on a raised keel; from the keel arise silica flaps, the conopea, and the valve face is depressed below the conopea to form conopeal canals. The valve surface is thus differentiated into three regions, in which the areola pattern may differ: the exposed valve face, the valve depression, and the exposed part of the keel wall between the conopeum and the raphe-sternum. Thirty-six morphological characters were used for cladistic analysis; these include a wide range of areola shapes on the three valve surfaces and the copulae – oval/circular pores, transapical slits, longitudinal slits, small pores fields, continuous pores fields, and larger quadrate pores closed by hymenes or cribra; the extent of the conopea along the valve; the presence of longitudinal ribs on the valve, conopea, or copulae; and the extent of internal development of silica below the striae. Cladistic analysis was 100% congruent with sequence interpretation. Comparisons were made with (1) type material of *Nitzschia angularis* and *N. spathulata* from BM and specimens of *N. martiana* from Guam, all of which require transfer to *Tholophora*; and (2) specimens of a freshwater taxon from Costa Rica, initially identified as *Nitzschia dissipata*; this species does not match descriptions of *N. dissipata* but clearly fit within *Tholophora* so will be described as *T. lasrucensis* n. sp.

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GENOMIC INSIGHTS INTO THE BIOSYNTHESIS OF PHOTOPROTECTIVE PIGMENTS IN PORPHYRA UMBILICALIS: CAROTENOIDS AND MYCOSPORINE-LIKE AMINO ACIDS

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As intertidal species, *Porphyra* is frequently exposed to excessive photosynthetically active radiation (PAR) and potentially harmful UV-light. Carotenoids are important for protection of the photosynthetic apparatus under excessive PAR, while mycosporine-like amino acids (MAAs) protect against UV-related damage. Here, we have explored the

genetic basis of the biosynthesis of carotenoids and MAAs in *Porphyra*.

By mining genomic and transcriptomic data, we recovered the almost complete gene repertoire required for carotenoid formation in *Porphyra*. Consistent with the accumulation of lutein, the *Porphyra* genome encodes two lycopene cyclases, one putatively catalyzing the formation of beta-ionone rings whereas the other likely has the epsilon-cyclase activity needed for lutein synthesis. It has been speculated that the epsilon-cyclases of red and green algae are orthologs. Our phylogenetic analyses, however, indicate that the epsilon-cyclases in Rhodophyta and Viridiplantae evolved independently by duplication of their respective beta-cyclase genes and that the two lycopene cyclase genes in cryptophyte algae originate from their secondary plastids of red algal origin.

In cyanobacteria, the biosynthesis of mycosporine-like amino acids involves the four genes MysA, MysB, MysC and MysD. *Porphyra* contains a gene encoding a MysA-MysB protein fusion that is also found in other intertidal red algae and in some dinoflagellates and heterokont algae, i.e., algae with secondary plastids of red algal ancestry. Although virtually absent from extant green algae, the MysA-MysB fusion is also present in chlorarachniophyte and euglenophyte algae with secondary plastids of green algal origin. Moreover, *Porphyra* and related red algae contain a gene encoding a MysC-MysD fusion protein, and in *Porphyra* and *Chondrus* the MysA-MysB and MysC-MysD fusion genes are located next to each other on opposite DNA strands. Conservation of the MAA gene cluster and the gene fusions suggest that this arrangement provides a selective advantage and efficient MAA biosynthesis for red algae experiencing high UV irradiance.

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COMBINING CULTURE AND METABARCODING FOR LITTLE KNOWN PHYTOPLANKTON GROUPS

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The ecological importance and diversity of pico/nano-planktonic algae remains poorly studied in marine waters, in

part because many are tiny and without distinctive morphological features. Two recent studies have demonstrated the power of mixing classical culture and phylogenetic analysis with novel massive metabarcoding approaches and shed light on the diversity and distribution of two important groups: bolidophytes and prasinophytes clade VII. Bolidophyceae form a monophyletic group phylogenetically close to the diatoms. We have confirmed, based on DNA sequence homologies of several gene markers, that the silica covered cells known as Parmales (genus *Triparma*) belong to the Class Bolidophyceae, a group characterized so far by naked flagellated cells (previously *Bolidomonas*). The global distribution of Bolidophyceae was assessed using environmental sequences available in public databases as well as a large 18S rRNA V9 metabarcode dataset from the Tara Oceans project. Prasinophytes clade VII are a group of pico-planktonic green algae (division Chlorophyta) for which numerous rRNA sequences have been retrieved from the marine environment in the last 15 years. A large number of strains have also been isolated but have not yet received a formal taxonomic description. Phylogenetic analysis of available strains using both the nuclear 18S and plastidial 16S rRNA genes demonstrates that this group is composed of at least 10 different clades: A1 to A7 and B1 to B3. Analysis of sequences from the variable V9 region of the 18S rRNA gene collected during the Tara Oceans expedition and in the frame of the Ocean Sampling Day consortium reveal that clade VII is the dominant Chlorophyta group in oceanic waters, replacing Mamiellophyceae which are most important in coastal waters.

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THE NAGOYA PROTOCOL ON ACCESS AND BENEFIT SHARING AND ITS IMPLICATION FOR ALGAL CULTURE COLLECTIONS AND THEIR USERS

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Algal culture collections serve as specified microbiological resource centers (MBRCs). They underpin and actively contribute to research, teaching and biotechnology with well-defined reference strains, associated data and broad expertise in isolation, identification and *ex situ* conservation. Since the Nagoya Protocol (NP) on Access and

Benefit Sharing (ABS) came into force in Oct 2014, greater attention on legal aspects of accession, exchange and use of genetic resources is required. MBRCs are main stakeholders to follow NP and legislations of source countries and raise awareness to users and depositors. Today 95 countries ratified the NP and 172 defined national ABS focal points. But many did not yet implement ABS legislation. However, collectors and bio prospectors must ensure legal clarity in how they can (or cannot) use genetic resources. Primary information about global NP and national legislations is provided on the CBD website (Convention of Biological Diversity) by the ABS Clearing House (<https://absch.cbd.int/>). Best practice guidelines are developed by institutions, research communities and national authorities. European Union Regulation 511/2014 implemented NP legislation for EU users including a 'register of collections'. Such registered collections prove to meet specific criteria. Their users automatically demonstrate due diligence when sourcing material (e.g. living algal cultures or DNA).

Registered collections apply standardized procedures for accessing and exchanging genetic material and related information in line with CBD and NP rules. These include extensive documentation for incoming and outgoing material. Accurate data on origin and deposition needs to be stored. Multiple ABS documents from source and import countries, permits and material transfer agreements along the deposition line may accompany the material. SAG currently expands its MySQL database and public catalogue to meet this demand and optimize user service. On national level SAG participates in a consortium of BRCs defining criteria for registration of collections.

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THE USE OF INTEGRATIVE TAXONOMY FOR IDENTIFICATION OF CRYPTOPHYTES – FIRST RECORDS FOR POLAND

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Recent studies on the cryptophytes proved that morphological and ultrastructural traits are not sufficient for taxonomy when considered separately without the support of molecular data. Most data about cryptophyte diversity in Poland are based on morphological determination of observed taxa and therefore are not taxonomically informative. The objective of this study was to recognize cryptophyte diversity in chosen ecologically different water bodies in Southern

Poland. Clonal cryptophyte cultures were established from the collected samples with the use of fluorescence-activated cell sorting. The differentiation of cryptophyte taxa was assessed using classical morphological data and molecular techniques, including sequencing of the partial nuclear LSU rDNA. The genetic marker was used as a barcode to identify the species that were revised molecularly. Preliminary results demonstrate the occurrence of *Cryptomonas obovoidea*, *C. phaseolus*, *C. curvata* and two *Cryptomonas* species that were not yet taxonomically revised. These are the first reports of cryptophytes in Poland confirmed with the use of integrative taxonomy.

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HYPERVARIABLE REGIONS OF 18S RDNA AS A TOOL FOR IDENTIFICATION OF AUTOTROPHIC EUGLENID SPECIES IN METABARCODING SURVEYS

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The application of molecular identification combined with next-generation sequencing (NGS) is an unprecedented opportunity to expand our knowledge about the biodiversity of eukaryotic microorganisms, their spatial distributions and their temporal dynamics. However several groups of microscopic algae, including autotrophic euglenids (*Euglena*), are known to be underrepresented in environmental sequencing surveys. This is most probably due to their highly variable and long 18S rDNA sequences (commonly used in metabarcoding as a DNA barcode for species identification) which may cause problems with amplification using universal eukaryotic primers. Therefore an individual approach to metabarcoding studies of this group, in particular the use of a customized DNA barcode marker, is necessary in order to identify species in environmental

samples. For autotrophic euglenids it is known that ITS sequences are extremely variable, and thus not suitable for species identification. We also rejected the COI gene due to the impossibility of a universal euglenid primers design. Therefore using a plethora of available methods we verified ten fragments of 18S rDNA (containing all hypervariable regions) as a potential DNA barcode. We found that variable regions V2-V3, V2, V4 and V8 of 18S rDNA are very efficient in identification of autotrophic euglenid species (efficiency above 95%) and two of them have the proper length for being used in Illumina based metabarcoding surveys. The reference database of more than 500 euglenid 18S rDNA sequences with verified taxonomic assignment was created using both sequences from GenBank and those newly obtained from environmental samples. We also designed primers that enable efficient PCR amplification of the chosen fragments and tested the performance of the proposed DNA barcodes in species assignment in silico and using mixtures of cultures.

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LINNEAN SYSTEMATICS IN THE AGE OF BIG DATA

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Carl Linnaeus (1707–1778) was the founder of modern taxonomic nomenclature. His most significant achievement was his universal system that designated every living plant and animal by a pair of Latin names for genus and species, in contrast to the existing increasingly lengthy polynomials. Linnaeus used his Latin binomials to catalogue well-known plants and animals, and new species as they were discovered during the golden age of exploration. Although a creationist, he was part of the Enlightenment, a disciple of Francis Bacon and emulator of Isaac Newton: he placed *Homo sapiens* in the Order Primates. He gave precise instructions on preparing herbarium specimens, emphasizing initial dryness, quick preservation in dry conditions, and use of fish glue; 16,000 specimens still exist. Plant names were not linked to the original ‘type’ specimens until the twentieth century, however, when the governance of the International Code of Botanical Nomenclature provided them as permanent reference points. The stable naming system that resulted is equally important today in the age of Big Data and will continue to be so into the future, as names (of eukaryotes) provide a portal to molecular, ecological and other biological data. This is literally true, as

Linnaeus's treatment of specimens preserved their DNA. Biologists are globally linked to the work of taxonomists by nucleotide sequences – in the new golden age of taxonomy! As high-throughput genomics lead to exponential growth of massive data sets, names help with the challenges of data mining. Background noise can swamp the signal, as in cosmology, so data provenance is essential and reality checks using digital images and real specimens must continue. The requirements (hardware, software and human) are daunting, but those responsible for future compatibility of systems seem confident that technology will continue to provide for enormous expansion in capacity.

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THE INFLUENCE OF SEDIMENT CHARACTERISTICS ON ESTUARINE BENTHIC MICROALGAE COMMUNITY STRUCTURE

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Benthic microalgae (BMA) are an important component of estuarine ecosystems, where they can account for more than 50% of total primary production. In New Zealand, however, we have a generally poor grasp of the species diversity and functional aspects of estuarine BMA taxa. The objective of this study was to assess the abundance of different taxa and their relationships with key benthic parameters. During the survey of the Avon-Heathcote estuary, one of South Island's most important coastal wetlands, we have collected and analyzed 32 sediment samples from various intertidal mud- and sandflat microhabitats. Each sample was analyzed for sediment grain size ratio, porosity, organics, and chlorophyll *a* content. Individual BMA communities were isolated by gradient centrifugation with colloidal silica Ludox HS-40 and afterwards the relative abundance of broad taxonomic groups and non-diatom species was quantified in those isolates. Precise diatom species identification and abundance quantification was done on permanent slides mounted with Naphrax. More than 50 diatom taxa together with 12 non-diatom species (cyanoprokaryotes, euglenoids, dinoflagellates) have been identified in

total. Naviculoid diatoms appeared to be the most abundant group in the studied sediments, however, they were often co-dominated by *Euglena obtusa* F. Schmitz along with filamentous and merismopedioid cyanoprokaryotes. The percentage of mud in the sediments was the most important factor affecting BMA diversity and biomass. Samples from muddy sand (<30% mud) had the highest chlorophyll *a* content and the most diverse BMA communities. In contrast, samples with a higher sand-to-mud ratio had lower algal biomass and contained more species-poor BMA communities.

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RELATIONSHIPS BETWEEN DIATOMS AND ENVIRONMENTAL VARIABLES IN INDUSTRIAL WATER BIOTOPES OF TRZUSKAWICA S.A. (POLAND)

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Diatoms are ecologically diverse and flourish in virtually every industrial water habitat. The heterogeneous nature of industrial environments make water monitoring challenging. We present relationships between diatoms and environmental variables from water biotopes in Trzuska-wica S.A. (Poland), which is one of the largest industrial plants in Europe producing cement, lime and asphalt mass. Water sites for algological, physical and chemical materials were sampled from March to September 2015: two reservoirs (stocked with fish and with technological water) and one drainage ditch. Analysis of water samples using X-ray fluorescence method (TXRF) revealed 17 elements: P, S, Cl, K, Ca, Ti, Cr, Mn, Fe, Ni, Cu, Zn, Br, Rb, Sr, Ba and Pb. A total of 36 diatoms were found but only three species in all sampling sites: *Cymatopleura radiosa*, *Navicula upsaliensis* and *Nitzschia angustata* were present. Some diatom species can be used as season indicators in industrial water biotopes. The relationships between diatoms and environmental variables confirm the positive correlation with the currently functioning industrial plant (despite the increased water temperature and large content of trace elements). Our results suggest that, despite heterogeneity in both diatoms and selected elements

in industrial waters, diatoms can be useful indicators of habitat conditions.

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MOLECULAR AND MORPHOLOGICAL INVESTIGATION OF COSMOPOLITAN DIATOM HANTZSCHIA AMPHIOXYS WITH REMARKS ON BIOGEOGRAPHY

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Until now, the diversity of representatives from the genus *Hantzschia* inhabiting of soils in Ukraine and European Russia was limited to the species *Hantzschia amphioxys*, *Hantzschia elongata* and *Hantzschia vivax* with some infraspecific taxa. We have studied the morphology, ultrastructure and phylogeny of 13 soil diatom strains belonging to *H. amphioxys* sensu lato using 28S rDNA and *rbcL*. We show that our strains contain five different species of *Hantzschia*, including three new to science.

Five strains were identified as *Hantzschia abundans*. We indicated an insignificant curvature of the raphe near its external central ends. Four of the examined strains were represented by different populations of *H. amphioxys* and their morphological characteristics fully correspond with the accepted isoelectotype and epitype. The main specific features of this species include 21–25 striae in 10 µm, 6–11 fibulae in 10 µm, 40–50 areolae in 10 µm and internal central raphe endings bent in opposite directions. Three new species were described based on differences in the shape of the valves, a significant excess of the dimensional characteristics, a smaller number of striae and areolae in 10 µm and the position of the internal central raphe ends. Based on the study of morphological variability and phylogeny of soil *Hantzschia*-species from different geographical locations we conclude that some sympatric populations of pseudocryptic taxa exist in the Holarctic.

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A NEW GENUS FOR SPECIES OF CORALLINE RED ALGAE ASCRIBED TO THE COSMOPOLITAN GENUS SPONGITES

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The non-geniculate coralline algal genera *Spongites* and *Pneophyllum* (Corallinales, Rhodophyta) are morpho-anatomically distinguished from each other by their tetrasporangial conceptacle roof formation. Based on modern molecular analyses, using a multi-gene analysis (LSU, SSU, psbA, COI) integrated with histological comparisons of type material and recently collected specimens, these genera were shown to be polyphyletic. A reassessment has led us to provide a new circumscription for the two genera, including the description of a new subfamily and a new genus to accommodate some species and specimens previously ascribed to *Spongites*. The new genus and *Spongites* both have Type 1 tetrasporangial conceptacle roof formation, in which the development is only from filaments peripheral to the fertile area. They may, however, be distinguished by a combination of characters, including the diameter of the tetrasporangial conceptacle chamber (>300 µm in *Spongites* vs. <300 µm in the new genus) and the tetrasporangial conceptacle roof thickness (>8 cells in *Spongites* vs. <8 cell in the new genus). Based on our findings, several species/specimens ascribed to *Spongites* from both South Africa and the Northeast Pacific will need to be transferred to the new genus. The findings of this study will more than likely also mean that a number of species/specimens from other geographic localities will also need to be formally transferred to the new genus.

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DEVELOPMENT AND ADOPTION OF A NEXT-GENERATION-SEQUENCING APPROACH TO DIATOM-BASED ECOLOGICAL ASSESSMENTS IN THE UK

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The UK has used diatoms for assessing the ecological condition of freshwaters since 1995 and, since the introduction of the Water Framework Directive, they have been used to fulfil obligations to assess “phytobenthos” in rivers and lakes in the EU. Assessments have hitherto been based on microscope counts of a few hundred individuals per sample, followed by calculation of the Trophic Diatom Index (TDI) as a basis for assessment of ecological status relative to reference pristine conditions. During the last 5 years, the UK Environment Agency has funded an R&D project to replace microscope-based counts by a DNA metabarcoding approach. This has involved (1) developing an *rbcL* reference library, evaluating candidate barcode regions within the *rbcL* gene, and establishing a bioinformatics pipeline to check, classify and identify NGS barcode reads; (2) comparisons of molecular and microscope-based assessments of community composition in 1337 paired NGS and LM samples; and (3) formulation of new NGS-based trophic diatom indices (TDI4-NGS and TDI5-NGS) and their calibration with respect to microscope-based TDIs. Despite the absence of many species from the barcode reference library and absence of a clear understanding of how *rbcL* copy number per cell varies within and among diatom species, NGS-based TDI indices provide assessments of ecological status for UK rivers that are comparable to microscope-based TDI assessments and usable for monitoring under the Water Framework Directive. Therefore, given the cost savings of the NGS approach, from 2017 onwards diatom identification and enumeration in England will move from light microscopy to a DNA metabarcoding approach using Illumina MiSeq next generation sequencing (NGS).

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EXPLORING THE STRUCTURE OF A PLANKTONIC ECOSYSTEM

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The northern Adriatic is a very convenient ecosystem to observe phytoplankton under a variety of different conditions. Steep gradients of nutrient concentration/availability in this shallow basin allow the observation of the same species under changing nutrient regimes on small temporal and spatial scales. After studying the *in situ* behavior of marine phytoplankton along the aforementioned gradients, we selected and isolated key species for *in vitro* experiments, where we analyzed their reactions to contrasting nutrient regimes. An imbalanced N/P ration in the basin and a resulting overall phosphate limitation appears to be a major driver and determining factor of the ecosystem. The reaction of key species to phosphate limitation and the resulting efficiency in niche formation and competition for ecological niches explain most of the *in situ* observations regarding abundance and spatio-temporal distribution of phytoplankton species. This study shows that *in vitro* characterization of phytoplankton species metabolic capabilities allows the prediction of their relative performance in *in situ* competition for resources.

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SKELTONEMA GREVILLEI, A REGULAR AND BLOOM FORMING DIATOM IN THE COASTAL WATERS OF THE ADRIATIC SEA

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Skeletonema grevillei Sarno et Zingone was described in 2005 from the original type material. Cells were delicate, 4–7.5 µm in diameter, and formed short colonies. The fulcrum processes in the terminal valves were open tubes having narrow tips with truncated or spiny margins. In the intercalary valves, they formed knuckle-like, generally 1:1 junctions. A scallop-work of silica bridges joined the bases of the processes. The type locality for this species is Hong Kong bay and it has also been reported in Japan, Australia and Muscat Oman. *Skeletonema grevillei* Sarno and Zingone was observed for the first time in the Adriatic Sea in 2014 during the autumn bloom, when it reached high abundances (5.4×10^6 cells L⁻¹). The bloom started in September with the peak in November/December and lasted until January/February. The highest abundances were found in the surface layer from 0–10 m depth. We observed colonies from 3 to 26 cells both in cultures and natural samples. The valve face was slightly convex and the pervalvar axis is generally longer than, or as long as, the cell diameter. The cell diameter was 5–19 µm. The IFPPs are rather long (8.5 ± 1.6 µm $n = 13$), each joining one IFPP of the adjacent cells (1:1 junction), with a thickening at the joint. Here we report in situ observations of *S. grevillei* with additional information on their morphological and genetic characteristics as well as ecology.

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PATTERNS OF DISTRIBUTION OF MACROALGAL DIVERSITY IN THE BALTIC SEA

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Baltic Sea is a basin with prominent set of different environmental gradients starting from south-north and west-east salinity gradient from almost marine to freshwater conditions along the 1400 km transect. Recently compiled checklist of macrophytes of the Baltic Sea includes 531 taxa of macroalgae, aquatic vascular plants, charophytes and bryophytes. Species diversity is distributed very unevenly

having largest species number (401) in the westernmost basin of Kattegat with pronounced marine conditions. Most species poor areas are those in the middle of the salinity gradient surrounding the central Baltic Proper while the easternmost and northernmost basins have slight increase in species number due to inclusion of some freshwater taxa. So far the salinity has been claimed to be the main ecological factor responsible for largescale patterns in species distribution of macrophytes in the Baltic Sea. At the same time most of the macrophyte species are very sensitive to quality of substrate and other properties of the microhabitat. We analyzed the distribution of macroalgal diversity along the Baltic Sea salinity gradient in relation to the diversity and quality of available habitats on detailed data from recently conducted inventory covering 17 Baltic Sea sub-basins. Applying different statistical and GIS techniques we used several habitat data layers from publicly available sources to describe patterns of macrophyte species diversity. Results demonstrated that in number of Baltic Sea basins macrophyte diversity depends rather on habitat availability and quality than direct influence of salinity gradient.

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VALVES AND AUXOSPORE MORPHOLOGY OF MARINE DIATOM ODONTELLA GRANULATA (ROPER) R. ROSS

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The morphology of the valve (frustule) and auxospore structure of the marine diatom *Odontella granulata* (Roper) R. Ross have been investigated. The living cell attaches to sand grains or detritus by mucilage extruded through the ocelli. The frustule is rectangular in girdle view, narrow elliptic to wide lanceolate in valve view. The two large ocelli present on the valve with clear elevations pointed in opposite directions. Plastids are discoid and numerous. The surface of the valve is covered with fine spines and spinules. The areolae are round foramina internally, and loculate externally. The two rimoportulae are situated

GRATOL-LEFT: PROGRESS AND CHALLENGES IN CHLOROPHYTA

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Green algae are diverse primary producers with essential ecological roles, and occupy a pivotal evolutionary position in relation to red algae, glaucophytes, and land plants. Green algae can be found in freshwater and marine habitats, desert soil crusts, tropical and temperate forests, thermal hot springs, and hypersaline ponds. The Green Algal Tree of Life project (GrAToL) is focused on resolving relationships among the phylogenetic breadth of green algae and plants, including many previously unstudied taxa, by compiling and analyzing large amounts of genomic data. Highlighted are the progress and challenges in resolving relationships among members of Chlorophyta that have so far resulted from this project. We illustrate non-monophyly of traditional taxonomic groupings, unexpected diversity among algae with simple morphologies, as well as surprising relationships in algae with complex morphologies. We reveal lineage-specific patterns in the evolution of organellar genomes, lateral gene transfer, and new genetic codes. New phylogenetically significant lineages, such as trebouxiophyte desert genera, families of Sphaeropleales, and the new class Palmophyllophyceae, are discovered as unusual habitats are surveyed and phylogenomic approaches are employed. Traditional morphological and ultrastructural traits have phylogenetic utility in some groups, whereas new traits are revealed in other lineages. Lastly, new phylogenetic methodologies for examining data combinability are developed.

midway between or near to the two ocelli. The rimoportula is externally extended as large hollow spine with sharp or forked exit tube at the apex, and it is simple lip-like and slit in the middle internally. The ocelli of valve surface densely are packed the hexagonal porelli. The cingulum consists of three to five girdle bands: a valvocopula and two to four copulae with the ligulae (the second to the fourth copulae). The mature auxospore of *Odontella granulata* is free, and similar to the vegetative frustles, with rather heavily silicified walls. The auxospore is formed perpendicular to the apical axis of gametangial cells. The initial valve is about three times longer than the vegetative (gametangial) valve. The auxospore is surrounded by incunabular strips, and consists of many transverse, longitudinal bands and many scales (incunabular scales).

WHAT PALEONTOLOGY CAN SAY ABOUT MODERN SILICOFLAGELLATES THAT PHYCOLOGY HAS MISSED

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Silicoflagellate study by paleontologists, as well as many biologists, has concentrated on the siliceous skeletons. These skeletal latticeworks are built of triple-junctions that suggest that an optimization of cellular surface area must be an important requirement during mitotic division. The silicoflagellate paleontology shows nine Cretaceous and at least seven Cenozoic genera that each have significant geologic records of five or more million years and wide geographic distributions, with two or three genera present in the Holocene. Phycologists have generally recognized only a single genus, in part due to an extraordinary variability observed in clonal cultures that is different from that seen in open oceans and the deep sea record. Recent paleontological and biological studies generally utilize three modern genera, *Dictyocha*, *Stephanocha* (which replaces the invalid *Distephanus*) and *Octactis*. The two primary skeletal morphogroups in modern oceans, *Dictyocha* and *Stephanocha*, have separate and distinct lineages that diverged in the Eocene (~40+ million years ago). A third skeletal morphogroup, placed in genus *Octactis*, differs from *Stephanocha* in possession of a sinistrally rotated apical structure and absence of pikes, may have an evolutionary history distinct from *Stephanocha* even though both have apical rings.

**NANTES CULTURES COLLECTION:
THE MICROPHYTOBENTHOS
BIODIVERSITY, A NEW AND ORIGINAL
RESOURCE FOR BIOTECHNOLOGY
APPLICATIONS**

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Estuaries and coastal areas are amongst the most productive marine ecosystems on earth. They are particularly valuable as habitats and feeding grounds for a variety of organisms (birds, shellfish, demersal fish and invertebrates), which often support the local economy. One of the major primary producing groups of these areas are microscopic algae that inhabit intertidal sediments, the microphytobenthos, forming dense biofilms at the sediment surface. The microphytobenthos provides an important energy source for the estuarine food web, has a central role in moderating carbon flow in coastal sediments and contributes to sediment stabilization.

In the past decade, microphytobenthos key roles in the functioning of coastal ecosystems began to be studied, the ecology, biology and physiology. But the assessment of useful metabolites remains little known. Microalgae which are usually studied and used for biotechnology applications are planktonic ones, rarely diatoms and never benthic, whereas diatoms largely dominate the global microalgal diversity. Microphytobenthos diversity remains a ‘secret garden’ with a powerful potential for research and development. The *Nantes Cultures Collection*, or NCC, referenced in the ‘*World Data Center for Microorganisms*’ as ‘NCC WDCM 856’ since 2002, and dedicated to the preservation, storage and assessment of microalgae, is the only collection worldwide hosting strains of benthic diatoms. With more than 200 strains, including more than 50 ‘blue diatoms’ and 40 genera, the NCC is a real conservatory of microphytobenthos diversity, mainly originating from the French Atlantic coast. Currently, the NCC is part of the consortium Atlantic Microalgae (AMI) supporting a PhD (Eva Cointet)

with the objective of determining lipid profiles of the NCC strains to supply new and original strains for biotechnology.

**MARCLIM: CLIMATE-DRIVEN RANGE
SHIFTS AND CHANGES IN ABUNDANCE
OF MACROALGAE ACROSS THE BOREAL/
LUSITANIAN TRANSITION ZONE.**

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Britain straddles the transition zone between cooler boreal and warmer Lusitanian biogeographic provinces, and many species of macroalgae reach their biogeographic range limits on or near to the UK coastline. The MarClim project has the most spatio-temporally extensive time-series for rocky shores globally, with data stretching back to the 1950s. MarClim surveys 100 sites annually around the coastlines of the UK and northern France, and has found that impacts of climate change on species distributions and population abundances are species-specific within marine macroalgae. Some species of warmer thermal evolutionary origins have shown increases in abundance during the 2000s, with a slow-down from 2010–2014, followed by a continuation in the increasing trend to date. In contrast, species of colder thermal ranges have shown a reduction in abundance across 22 degrees latitude of their distributional range, and contractions in the trailing range edge since the onset of anthropogenic climate change in the 1980s. Differential responses have resulted in changes to community structure across this transition zone.

**HEAT STRESS MEMORY
IS RESPONSIBLE FOR ACQUISITION
OF THERMOTOLERANCE IN THE RED
SEAWEED BANGIA FUSCOPURPUREA**

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Land plants generally acclimate to stressful conditions and acquire stress tolerance by retaining memory of the stress. Since red seaweeds live at the intertidal zone where environmental conditions are frequently changeable, it is

possible that red seaweeds also have an ability to acquire stress tolerance via stress memory. We therefore studied heat stress response and thermotolerance in the red seaweed *Bangia fuscopurpurea* to understand whether aquatic plants are endowed with stress memory. Since the limiting growth temperature of *B. fuscopurpurea* was around 30°C, we first examined an ability of acquisition of heat stress tolerance by pre-heating (priming) at 28°C for one week and subsequent cultivation at 32°C for one week. Results indicated that priming triggered acclimation to heat stress for survival at lethal temperature, whereas no viability was observed when seaweeds were directly transferred from 15°C to 32°C. Thus, *B. fuscopurpurea* can acquire thermotolerance against lethal high temperature by pre-exposure to non-lethal high temperature. Next, involvement of stress memory in acquisition of heat stress tolerance was examined by incubation of the primed seaweed at 15°C for various durations and subsequent cultivation at 32°C for one week. These experiments indicated that thermotolerance against lethal high temperature was kept at least for 5 days, although the strength of thermotolerance was gradually reduced. Thus, *B. fuscopurpurea* can memorize non-lethal heat stress by priming and can maintain the primed state at least for 5 days. Priming at 28°C prevented saturation of membrane fatty acids during subsequent 32°C cultivation, although a large increase in saturation rate was observed in non-primed seaweeds at 32°C incubation. Therefore, it seems that changes in cellular physiological state caused by heat stress memory reduces the sensitivity of *B. fuscopurpurea* to lethal temperature and this ability is maintained for 5 days by heat stress memory under normal culture conditions.

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SOMATIC CELL FUSION IN A RED ALGA *GRIFFITHSIA MONILIS* IS MEDIATED BY TWO DIFFERENT SIGNALLING MOLECULES

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When a cell in the red algal filament is wounded, a cascade of cellular event takes place in the adjacent cells. The dead cell is replaced through the somatic cell fusion between a repair-shoot cell and repair-rhizoid cell produced from adjacent cells. During this repair process, repair cells show

attracted growth towards each other. Rhodomorphin released from the repair-rhizoid cell has been reported to be responsible for this attracted growth between repair cells. However, our time-lapse videography showed that this attracted growth needs an additional signalling molecule in addition to rhodomorphin, suggesting two different signalling molecules are involved in this process. The genes specifically expressed during the somatic cell fusion were isolated from the transcriptomes of *Griffithsia monilis* and their expression profile was studied using qPCR. Predicted gene model of the wound-healing process of *Griffithsia monilis* will be discussed.

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A NEW MECHANISM OF VIRAL IMMUNITY IN MARINE PHYTOPLANKTON

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Mamiellophyceae is an ecologically important class of green microalgae in the ocean, and Prasinoviruses infecting them are probably the most numerous kind of large DNA viruses of eukaryotes in the sea. Surviving viral attack is essential for any species to avoid its irreversible removal from the ecosystem, and microbes this must involve the resistance or susceptibility of individual cells. The co-existence between viruses and their hosts has led to the evolution of complex viral attack and host defence strategies. We showed that Mamiellophyceae, as with all phytoplankton populations, are largely controlled by these hugely diverse populations of viruses. We sequenced many of them and showed that mechanisms of resistance operate in these species. Virus-resistant cell lines arise in many independent cell lines during lytic infections. In these resistant lines, there was over-expression of all genes in more than half of an unusual chromosome, and karyotypic analyses showed physical rearrangements of this chromosome. It has an unusual genetic structure whose equivalent is found in all of the sequenced genomes in this ecologically important group of green algae. We propose that structural instability of this chromosome is under the control of viral infection and provides viral immunity.

EXPLORING BACTERIAL DIVERSITY IN INVASIVE GREEN ALGAE.

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Siphonous green seaweeds are among the morphologically most complex algae and also among the most notorious invasive species in many parts of the world. Their ecological success has repeatedly been linked to their association with endo- as well as epiphytic bacteria. The competitive advantage of invasive species may also be at least partly shaped by associated microbes. Indeed, recent studies revealed rich associated bacterial communities. However, little is known about their functional diversity as well as the principles underlying their assembly. To address this, a 16S rDNA metabarcoding approach was applied to analyse the bacterial diversity of epi- and endophytic bacterial communities associated with native and invasive species of *Caulerpa*, namely *C. prolifera* and *C. cylindracea*. Natural populations of both species were sampled from three regions along the Turkish coastline of the Aegean sea. To further explore bacterial diversity and assembly, in situ experiments were performed focussed on changes in bacterial community structure due to temperature stress as well as increased nutrient load. This study aims to elucidate the natural microbiome structure of both the native and invasive *Caulerpa* species and their response to alterations in environmental abiotic conditions.

BIODIVERSITY OF THE GENUS *HASLEA* AND POSSIBLE APPLICATIONS

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The genus *Haslea* is a taxonomic unit of pennate diatoms referring to fusiform or lanceolate cells with a frustule made of two valves, each presenting straight striae, both transversally and longitudinally. Characteristically, *Haslea* frustule valves present a totally different aspect when comparing their external and internal surfaces. The former presents continuous longitudinal fissures, the latter is perforated by areolar, square to rectangular openings, forming grate-like bars. These two layers are held together and assembled in a typical sandwich-like structure. The genus *Haslea* type species is *H. ostrearia*, a tychopelagic/benthic/epiphyte organism that produces marennine, a water-soluble blue pigment responsible for the greening of oysters in Western France. Marennine is also a bioactive molecule, and blue *Haslea* have a high potential for use in existing oyster farming, for the production of pigments and bioactive compounds with natural antibacterial or cytostatic properties, and for novel applications as an industrial colouring agent. The H2020 project *GHaNA* (The genus *Haslea*, new resource for biotechnology and aquaculture) has been funded to explore the genus *Haslea* for blue biotechnology applications. This project is a collaborative research program based on a partnership between academic and non-academic partners. It will determine the biological and chemical diversity of *Haslea* diatoms and will develop mass-scale production processes, to achieve viable industrial production of biomass and associated high-value compounds, including terpenoids, marennine-like pigments and lipids. Moreover, silica skeleton wastes from frustules could be exploited as inorganic biocharges in the formulation of new elastomeric materials. These objectives will be achieved through fundamental and applied research to isolate fast-growing strains of *Haslea*, optimise their growth environment and cultivation system (*ad hoc* photobioreactors) to increase the production of marennine and other high-value compounds, develop blue biotechnology specifically applicable to benthic microalgae (biorefinery approach), and to explore the potential for industrial exploitation of colouring and bioactive compounds in commercial aquaculture, food production, cosmetics and health.

GENETIC DIVERSITY AND CONNECTIVITY IN A COMMON RED ALGA, *GRACILARIA SALICORNIA* (GRACILARIACEAE, RHODOPHYTA) ALONG THE COASTS OF THAILAND

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Understanding the pattern of genetic diversity and spatial connectivity is essential for promoting proper management and conservation strategies for marine species. A marine red alga, *Gracilaria salicornia*, is currently common in Southeast Asian waters, and is becoming increasingly incorporated into food for human consumption or animal feed in Thailand. In this study, we analyzed the intraspecific genetic diversity and population connectivity of *G. salicornia* from across the east (the Gulf of Thailand) and west coasts (the Andaman Sea) of Thailand based on plastid-encoded RuBisCo spacer sequences. Our results revealed low genetic diversity within this species, and only six haplotypes were recognized along the coasts of Thailand. Our molecular data indicated that algal populations from the Andaman Sea showed higher levels of genetic diversity than populations from the Gulf of Thailand. We also found genetic differentiation between the two coasts: two haplotypes restricted to the Gulf of Thailand and the other four haplotypes to the Andaman Sea. This study suggests the differences in genetic diversity and distribution of *G. salicornia* population between the two sides of the Thai-Malay peninsula, and this genetic discontinuity is likely to be related to the oceanic circulation pattern in the region.

SHEDDING LIGHT ON THE DARK – A COMPREHENSIVE DE NOVO TRANSCRIPTOME OF *COSMARIUM CRENATUM*

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The Zygnematophyceae are the sister group to the Embryophyta and therefore an important model system in early land plant evolution. Many studies focused on photosynthetic performance, ultrastructure, cell biology and growth patterns in this group. So far, neither a genome nor a comprehensive cDNA library has been published.

In order to study gene expression under various conditions, a polar isolate of *Cosmarium crenatum* was exposed to more than 40 abiotic stress treatments including darkness, high PAR, UVR and temperature. To monitor the physiological status, photosynthetic efficiency and growth parameters were measured. To cover initial, short-term and long-term acclimation, sampling was done in three different time intervals (1 hour, 1 day and 1 week). A normalized reference transcriptome has been established by Illumina HiSeq sequencing in 300 bp paired-end modus, consisting of 28,992,600 reads. A total of 141,711 assembled transcripts were generated, containing 54,389 trinity genes. We were able to annotate 58,826 gene functions via blastx. The reference transcriptome displays a high functional genomic coverage, including all major metabolic pathways i.e. carbohydrate, amino acid, lipid and nucleotide metabolism. Furthermore, pathways associated with stress responses of land plants were found e.g. ROS metabolism.

To investigate transcriptomics underlying long-term darkness exposure, an Illumina HiSeq approach was applied. Under darkness compared to low light conditions 4,138 genes were down-regulated and 3,767 up-regulated. Strongest down-regulation was observed for genes encoding components of lipid metabolism and photosynthesis. Interestingly, genes involved in phototropism were induced under darkness. The data indicates that *Cosmarium crenatum* undergoes several transcriptional changes under long-term darkness conditions. The dataset provides new insights into the molecular acclimation process of Zygnematophyceae to dark conditions, with important findings in early land plant phototropism development.

AUTOPHAGY IS A CENTRAL CELLULAR PROCESS IN THE INTERACTION BETWEEN BROWN ALGAE AND PARASITIC WATER MOULDS (OOMYCOTA)

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Anisolpidium ectocarpii, an obligate oomycete pathogen of brown algae, was subjected to ultrastructural investigation towards understanding its interaction with the kelp *Macrocystis pyrifera* at a cellular level. Electron microscopy revealed that successful penetration into the host leads to the development of a walled *Anisolpidium* syncytium, which in optimal growth conditions fills the entire volume of the cell wall and accumulates lipid globules. Sometimes, and likely because of starvation, the *Anisolpidium* syncytium shrinks inside the space delimited by the cell wall, conspicuous plasmalemma invaginations appear in the pathogen's periplasmic space, and some spore initials undergo autophagy, in a response that probably aims to rescue the development of a limited number of pathogen spores. In extreme cases, entirely abortive *Anisolpidium* syncytia show extensive evidence of uncontrolled autophagy despite the presence of lipid reserves. Besides cell wall reinforcements, infected algal cells exhibit many digestive vacuoles, some of them with *Anisolpidium* structures. Indeed, in vivo staining with monodansylcadaverin showed that over time, autophagy is induced in both the oomycete and the host. Autophagy, therefore is i) induced by the algal host as a local and possibly systemic defence to mobilise energy required to mount defences, and/or directly kill the intruder; ii) induced by the pathogen to adjust its sporulation to the level of resources it can retrieve from its host. We further hypothesise that iii) both the host and the pathogen may hijack their counterpart's autophagic process: depending on the winner of the interaction, systemic algal autophagy may provide extra resources to the pathogen, or a last line of defence for the alga to induce the suicide of its parasite. Complementary observations performed on two other oomycetes *A. rosenvingei* and *E. dicksonii* and another alga, *Pylaiella littoralis*, suggest that this response is at least partially conserved with other brown algae and oomycetes.

LAMINARIOCOLAX AECIDIOIDES AS THE CAUSATIVE AGENT OF MALFORMATIONS IN LESSONIA BERTEROANA FROM ATACAMA: DNA BARCODING, ULTRASTRUCTURE AND FIELD PREVALENCE

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Lessonia berteroa is the most strongly exploited seaweed in Chile and the Americas. It is frequently overharvested, which is detrimental for coastal communities in northern Chile. Concomitant to these abundance problems, several *L. berteroa* stocks were found to bear tumour-like deformations of different size (0.5–5 cm), colouration (yellowish to dark brown) and morphology (flat, circular, thorn-like or deformed galls). Inspection of these galls by means of histology, culturing and DNA barcoding showed that all of them were associated with the brown algal endophyte *Laminariocolax*. Light microscopy revealed the presence (but low prevalence) of both pigmented and colourless endophytic filaments inside the galls. After gall excision and cultivation in culture medium, brown algal filaments emerged. DNA barcoding in both galls and filamentous isolates from these galls (strains Lx TCE and Lx CCE) revealed the presence of *L. aecidioides*, using three different markers (nrDNA ITS1, mtDNA COI, cpDNA rbc L). They clustered with the endophytes *L. macrocystis* previously found in southern Chilean *Macrocystis* and *L. eckloniae* from South African *Ecklonia*. Electron microscopy showed cellular modifications of the *Laminariocolax* endophytic network in contrast to free-living isolates (e.g. organelle and cell wall reductions, larger area of plasmodesmata in undulated contacting cell walls). The *Lessonia* galls were characterized by hyperplastic, cell-wall-disrupted medullar cells. A demographic survey, supported by a generalized linear model, revealed that galled specimens are predominantly present in summer, and in adult individuals larger than 1.5 m.

ATTACHED DIATOMS ON THE CARAPACE OF GREEN SEA TURTLES (*CHELONIA MYDAS*) FROM OGASAWARA ISLANDS OF JAPAN

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Although sea turtles have long been familiar to marine biologists, many aspects of their ecology remain unaddressed. The present study is the first on the epizoic diatom community covering the carapace of green sea turtles (*Chelonia mydas*) from Japan. And the first describing diatoms living on sea turtles in general, with the primary objective of providing detailed information on turtle epibiotic associations. Samples of turtle carapace including the associated diatom biofilm were collected from Ogasawara Islands (24–27°N 140–142°W), Japan, in April and July 2016. A complex diatom community was present in every sample. In total, the following 12 diatom taxa were found there: *Achnanthes elongata*, *Amphora* sp., *Hyalosira tropicalis*, *Hyalosira* sp., *Mastogloia obliqua*, *Navicula* sp., *Nitzschia* sp. 1, *Nitzschia* sp. 2, *Poulinea lepidochelicola*, *Poulinea* sp., *Rhopalodia pacifica* and *Tabularia parva*. *Achnanthes elongata* was found in all samples. *Poulinea lepidochelicola* was found on 12 out of 19 turtles. These two species have also been recorded from the carapace of the olive ridley sea turtle (*Lepidochelys olivacea*). Together with the results of this research, it is suggested *A. elongata* and *P. lepidochelicola* commonly grow on the carapace of sea turtles.

COMPARISON OF GENOME BETWEEN OPPOSITE MATING TYPES OF PSEUDOSTAurosIRA TRAINORII

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Diatoms are unicellular algae with a unique life cycle. Because of the presence of a rigid silica cell wall, diatom cells progressively reduce their size during repeated cell

divisions. To avoid cell death by size reduction, diatoms restore maximum cell size via sexual reproduction, a process which is not understood well with respect to the molecular mechanisms of its regulation. Sexual reproduction in the araphid pennate diatom *Pseudostaurosira trainorii* is readily controllable in the lab using strains belonging to opposite mating types. In this species, males produce a gamete with a special flagellum-like structure called a thread, this structure is sticky and is used to search for and fuse to immobile female gametes by way of a spinning motility. Furthermore, *P. trainorii* is one of a few diatom species in which the presence of sex pheromones is proven.

To reveal the mechanism of sex determination and the molecular regulation of sexual reproduction, we are currently sequencing whole genome of *P. trainorii* in both male and female strains. In this presentation, we show our preliminary results from sequencing, including the nearly complete genomes of the chloroplast and mitochondrion. Between the male and female strains, there are several structural variations (short insertion/deletion) in both organelles. These variations enable us to determine male/female marker regions. Using the crossing experiment and this genetic information, we will determine the mode of organellar genome inheritance. This will be the first attempt to target two organelles at the same time in the same diatom species. We also compare the organellar genomes of *P. trainorii* with other diatoms.

LIFE HISTORY OF TWO MORPHOLOGICAL VARIANTS OF HYPNEA PSEUDOMUSCIFORMIS (GIGARTINALES, RODOPHYTA)

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H. pseudomusciformis Nauer, Cassano & M.C. Oliveira is a new species from the *H. musciformis* (Wulfen) J.V. Lamouroux complex described for the Brazilian coast. In Brazil, *H. musciformis* and *H. nigrescens* Greville ex J. Agardh are morphological variants of *H. pseudomusciformis*, and its taxonomy is quite problematic and confusing, due to the

fact that these algae possess a simple morphology, often influenced by environmental conditions. In this study, individuals of both morphological variants of *H. pseudomusciformis* were collected, and cultivated in the same unialgal culture conditions (sterile seawater adjusted to 21 psu and enriched with von Stosch growth medium, 14 h light: 10 h dark, 24 ±1°C, 70 ±5 μmol photons m⁻² s⁻¹). The life history of *H. pseudomusciformis* from both morphological variants, “*H. nigrescens*” and “*H. musciformis*”, was completed in 121 days. Tips of a tetrasporophytic specimen were incubated in the standard culture conditions in Erlenmeyer vials with 500 ml of medium. Tetrasporophytes produced tetrasporangia in 30 days. Released tetraspores germinated and around 15 days later, an erect cylindrical axis developed from basal disk. Later, erect axes became sparsely branched. Secondary erect axes were formed from a basal disk. At the age of 45 days, spermatangial conceptacles and cystocarps could be observed. Carpospores germinated and originated new tetrasporophytes. The growth rates (GR) of both variants were available during a period of four weeks. “*H. musciformis*” showed a higher GR than “*H. nigrescens*”, especially in the last week. This is the first time the *Hypnea* life history was completed in vitro.

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SPECIES DILEMMA: AN CASE STUDY ON HYPNEA MUSCIFORMIS (GIGARTINALES, RHODOPHYTA)

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Hypnea musciformis is a red macroalgae widely distributed in tropical and sub-tropical coasts around the world and is of great economic importance as source of industrial production of carrageenan. DNA barcode marker 5P-COI and the plastid *rbcL* gene, in addition to morphological studies, were used to investigate the species on the coast of Brazil and to compare it with specimens from other countries, including the type locality in Italy. This study led to the description of a new species *Hypnea pseudomusciformis* Nauer, Cassano & M.C. Oliveira for the coast of Brazil to accommodate three morphologically divergent species occurring on the Brazilian coast, *H. musciformis*, *H. nigrescens* and *H.*

valentiae. In this work, algae material was isolated and has been kept in unialgal culture (photoperiod of 14 h, 25°C and 30 ±10 μmol photons m⁻² s⁻¹) in the germoplasm bank, and in vitro breeding tests were conducted between specimens with the typical morphology for “*H. nigrescens*” and “*H. musciformis*”. Three male gametophyte and three female gametophyte individuals each were used and cystocarps were present in all gametophytes, confirming the molecular data that show that those algae belong to the same species. The identification of specimens based only on morphological characteristics proved to be unsatisfactory mainly due to phenotypic plasticity in this species. The technique of DNA barcoding was essential for the identification and definition of species, revealing scenarios that would go unnoticed by using only morphology.

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TAXONOMIC STATUS OF ACARPOSPOROPHYTUM IN THE GENUS BATRACHOSPERMUM (BATRACHOSPERMALES, RHODOPHYTA)

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Acarposporophytum was described as a subgenus of *Batrachospermum* with a single species (*B. brasiliense*) from southeastern Brazil. The species is unique among the members of the order in its lack of carposporophyte, with the tetrasporophytic (‘Chantransia’) stage developing directly from the fertilized carpogonium onto the gametophyte. In a general revision of the order, it was reduced to the section level of *Batrachospermum* with the remaining sections: *Aristata*, *Batrachospermum*, *Gonimopropagulum*, *Helminthoidea*, *Macrospora*, *Setacea*, *Turfosa* and *Virescentia*. This taxonomic scheme excluded the former sections *Contorta* and *Hybrida*, raised to the genus level as *Kumanoa*. *Batrachospermum* was provisionally accepted as paraphyletic and to resolve the infrageneric relationships, sections *Helminthoidea* and *Setacea* has recently been raised to the genus level. Thus, a tendency to raise the monophyletic groups within *Batrachospermum* to genera has emerged, although an alternative scheme to consider larger groups as genera has also been proposed. The taxonomic status of *Acarposporophytum* was evaluated using molecular evidence (sequences of *rbcL* and SSU rDNA) in combination with currently accepted morphological characters. The species

was positioned in a long branch with high divergence levels in comparison to the closest groups for both molecular markers. The divergences between *Acarposporophytum* and the closest groups were the same or higher than those accepted to distinguish genera. Based on these findings and following the concept of splitting smaller monophyletic groups to resolve the paraphyly, *Acarposporophytum* is raised to genus. This proposal reflects its unique characteristic of lacking the carposporophyte. The phylogenetic relationship of the genus with other groups in the Batrachospermales is still not resolved, with different sister groups found for each marker: Aristata and Macrospora based on rbcL and SSU, respectively. To determine the positioning of *Acarposporophytum* in the Batrachospermales, as well as to clarify the intraordinal classification, a phylogenomic approach is recommended.

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RETICULATE EVOLUTION IN FUCOID SEAWEED RADIATIONS

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Evolutionary inferences typically assume species-level monophyly, i.e., that 1) species represent genetically and reproductively independent lineages and 2) that reconstructed gene trees accurately reflect species trees. Polyphyly (as detected by gene/species conflicts) is not uncommon among marine taxa, however, suggesting that incomplete isolation and reticulate evolution often accompany marine lineage's diversification. Here we review selected case studies from brown algae radiations. *Fucus* and *Pelvetiopsis* (Fucaceae, Phaeophyceae) are two sister genera of intertidal, canopy-forming seaweeds with diversification centres in the N Atlantic and the NE Pacific, respectively. They are poor dispersers and commonly exhibit strong genetic structure, and both form multispecific intertidal assemblages where opportunities to hybridize are presumably high. Using phylogenetic and phylogeographic approaches we identify multiple instances of mtDNA introgression (*Fucus*) and of hybrid (polyploid) speciation (*Pelvetiopsis*). We show that organellar introgressions can be associated with climatic shifts, involve multiple species pairs and range geographically from local to complete replacement of native cytoplasmic genomes. Allopolyploidy is apparently rarer among seaweeds and marine organisms in general but identified cases appear to be linked with increased evolutionary success. Reticulate evolution may also be relevant

but overlooked in other, less well studied marine radiations. Our studies with fucoids highlight the importance of an adequate genomic and geographical sampling to recognize regional introgression and hybrid speciation when reconstructing the evolutionary histories of hybridizing marine species.

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UNDERSTANDING VULNERABILITY AND THREATS TO MACROALGAE IN THE CONTEXT OF INSULAR RARITY AND ENDEMISM IN A REMOTE ARCHIPELAGO, NEW ZEALAND

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The New Zealand Threat Classification System is a distinct departure from the international IUCN Red List categories used in many parts of the world for undertaking threat assessments. The development and use of the New Zealand system reflects a number of issues, particularly that it more accurately reflects the nature of insular rarity within the New Zealand archipelago. An initial assessment of threatened marine macroalgae was made in 2005 considering 60 exemplar taxa, and an unpublished update was carried out in 2009 with 260 species considered. In 2017 the status of the known New Zealand macroalgal flora (915 taxa), including taxa that are named and those recognised but unnamed, is being evaluated, using the criteria and procedures outlined by Townsend et al. (2008). Our previous analyses of numbers of specimens available for study, and interpretation of species distributional ranges and phenology, provide background for the high number of taxa that have been assigned the category Data Deficient. A summary of the current status and the particular issues facing categorisation of the macroalgal flora are presented, as well as examples of species whose threat status has changed since 2005.

ESTABLISHING A CULTURE COLLECTION OF THE DIATOMS FROM CẦN GIÒ MANGROVE BIOSPHERE RESERVE IN VIETNAM

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The Cầ̀n Giò Mangrove Biosphere Reserve (CGMBR) is a protected wildlife sanctuary listed by the UNESCO, with a high ecological importance. Some studies have been recently dedicated to the diatoms found in the mangrove's sediments and the benthic specimens sampled in the mud flat or the roots of trees. Our purpose is to establish a culture collection of the diatoms from the mangrove, to study their biodiversity by performing taxonomical studies with the most accurate tools, i.e. Scanning Electron Microscopy and molecular barcoding. The isolation and cultivation of diatoms in CGMBR is currently being conducted in Saigon University, to establish monospecific and monoclonal cultures. This process involves many steps and can entail prolonged periods of time. After 3 months, five strains of diatom are now isolated and placed in *f/2* medium, 24 ±1 °C, light/dark cycle: 12/12 for growth, and many others are under the process of isolation. Special attention will be paid to the study of species belonging to the Naviculaceae family, especially the genus *Haslea*, in the frame of the H2020-MSCA-RISE-2016 GHaNA 734708. In addition to the study of biodiversity and cryptic diversity of mangrove diatoms, this culture collection will be a valuable asset to the many aquaculture installations in the surrounding area, principally black tiger shrimp (*Penaeus monodon*), mud crab (*Scylla serrata*) and oyster (*Crassostrea* spp.) farming.

LIVING IN THE BOUNDARY LAYER OF KELP BLADES: REFUGE FROM OCEAN ACIDIFICATION OR TRAINING FOR HARSH CONDITIONS?

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Seaweeds are able to modify the chemical environment at their surface, in a micro-zone called the diffusive boundary layer (DBL), via their metabolic processes controlled by light intensity. Depending the thickness of the DBL, sessile invertebrates such as calcifying bryozoans living at the surface of the blades can be affected by the chemical variations occurring in this layer. Especially in a context of ocean acidification, these microhabitats might be considered as a refuge from lower pH during daylight, as photosynthesis temporarily pushes the pH higher than in the surrounding seawater. We characterized the thickness of the DBL in different pH conditions (current, pH 8.1 and a condition predicted for the end of the century in the context of ocean acidification – pH 7.7) and seawater flow (slow: 0.5 and fast: 10 cm s⁻¹) on *Ecklonia radiata* blades, the main kelp in the southern hemisphere. Oxygen profiles from the blade surface to the surrounding seawater were also measured with microsensors in both bare blades and blades colonized by the bryozoan *Membranipora membranacea* to assess the influence of epiphytes on the DBL structure. As expected, the DBL was thicker in slow than in fast flow. Surprisingly, the presence of bryozoans on the blades increased the DBL by creating their own DBL in addition to the kelp one. The gradient of oxygen in the DBL was affected by the presence of bryozoans and the mainstream pH in different ways, particularly in slow flow. As pH is mainly influenced by oxygen dynamics in kelp blades, oxygen variations at the algal surface led to broader pH variations between light and dark on blades with bryozoans, which shifted towards higher values in ocean acidification condition. These results show that living in the DBL of the kelps can constitute a refuge from ocean acidification or a training for harsh conditions for calcifying organisms living there, particularly in slow flow conditions.

THE EFFECT OF SEAWEED AQUACULTURE COOPERATIVES ON SUSTAINABLE LIVELIHOODS IN MALAYSIA

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Seaweed farming in Malaysia provides a source of livelihood for more than 1,000 families. The industry was limited by many governance, economic, environmental, technological and sociocultural challenges. A seaweed aquaculture cooperative – the Seaweed Cluster Project (SCP) – was introduced by the Malaysian government in 2012 to overcome these challenges and transform seaweed aquaculture into a commercial-scale venture. Using a mixed-methods approach we investigated the overall effectiveness of the cooperative ethos of the SCP, focusing on the sustainability of seaweed commercialisation and livelihood impacts. Clustering individual farmers into cooperative groups using a top-down interventionist approach resulted in fewer positive and more negative impacts on seaweed communities. The SCP farmers were positive when they received government assistance but were negative on the poor capacity building provided by the fisheries department. Tellingly, migrant farmers out of the SCP were the more successful seaweed farmers. The SCP is a top-down initiative that exerts total control over the project, rather than involving the communities in decision-making, and this hindered progress. There was poor coordination between levels of governance, weak market mechanisms, and un-integrated community development. Greater success could have been achieved with better fisheries governance and more understanding of local needs in the early stages of the policy-making process. With more initial research, the government would have had better information about whether seaweed farming had a significant future in the country. Lessons about economic interventionism can be learned from the SCP and might improve its fisheries governance. As such, lessons might also apply elsewhere in the world where cooperative approaches to seaweed cultivation are considered as part of the process to transition from seaweed cultivation and agronomy. This study has also highlighted key areas where the SCP management system

would have benefitted from a greater scientific input, such as disease mitigation and maximising plant productivity. Keywords: Aquaculture, Governance, Livelihoods, Food security, Coastal communities

CONSERVATION OF PHENOTYPIC PLASTICITY ALONG THE STABLE ENVIRONMENTAL GRADIENTS OF THE BALTIC SEA

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The Baltic Sea, a young habitat in geological terms, is characterized by strong climatic and salinity gradients. Especially salinity has been shown to trigger adaptation processes which may lead to speciation. However, as we speak of an ongoing event, the distinction between ecotypes and “real species” is difficult and depends not only on the applied species concept.

This presentation deals with the problem to identify taxonomic levels of sub species. Charophytes are a detailed example for ongoing speciation processes in algae. The presented distribution data display several members of the morphologically variable *Chara hispida*-group (e.g. *Chara horrida*) as candidates for species that may have developed in the Baltic Sea. However, genetic separation within this group is hard to determine by the currently available marker sequences. If the morphology of these brackish-water species are genetically pre-determined or caused by site-specific acclimation, is unknown. To address this question environmental stressors were used to alter the morphology of taxa belonging to the *Chara hispida*-group. For example, studies mention *Chara baltica* and *Chara liljebladii* as ecotypes acclimated to different irradiance regimes that convert them into each other. In our experiments, the conversion of morphology by irradiance was not successful and the question about their taxonomic rank remains even if these taxa cannot be separated genetically (neither by AFLP nor by sequencing).

We reasoned that the majority of current genetic markers is of limited value for the identification of sub species taxonomic ranks in cases of ongoing speciation. They are almost unrelated to functional or ontogenetic processes as speciation – a consequence of persistent acclimation – will target other sites in the genome. For future investigations, the combination of both ontogenesis and functional genes can help to better understand the evolution of species.

SMALL ARTIFICIAL POND AS A HOT SPOT FOR *ARTHROSPIRA JENNERI* (CYANOBACTERIA) – ENVIRONMENTAL AFTER EFFECT OF MASSIVE BLOOMING

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In the eastern part of the Łódź city (Central Poland) a municipal stream loaded with industrial pollution crosses the urban and suburban area. While performing a field survey to assess the ecological status of the stream, small parts of cyanobacteria thalli floating freely downstream were noticed. These thalli were formed by *Arthrospira jenneri* trichomes. It was later discovered that in the upper part, the stream flows through a small artificial pond – the Olechowska pond, where a massive benthic bloom of *A. jenneri* has been recorded.

The bloom was noticed in September 2016, when the bottom of the pond was completely covered with cyanobacteria thalli. Since then, with the exception of winter when no field survey have been performed, the bloom was recorded as sustained. In May 2017 the large lobes of thalli breaking off from the bottom of the pond were easily visible near the surface of water.

Investigations carried out along the stream and other artificial ponds connected with the stream revealed that the Olechowska pond is the only habitat – a hot spot – where *A. jenneri* grows.

Such massive and persistent growth influenced the hydrochemical conditions of the pond. The stream above the pond was characterized by strongly alkaline conditions (pH 9.1–9.3), high conductivity (1,348–1,180 μ S) and concentration of: 0.72 mg L⁻¹ ammonium; 0.98 mg L⁻¹ nitrates; 0.01 mg L⁻¹ phosphates. Water of the Olechowska pond was slightly alkaline (7.2 pH), with a low conductivity (177 μ S) and 0.08 mg L⁻¹ phosphates. Ammonium and nitrate ions usually were not detected (below spectrophotometric detection limit 0.005 mg L⁻¹) except after intensive rainfalls when nitrate concentration increased to 0.53 mg L⁻¹ as a result of surface runoff from farm fields. It is highly probable that this is a result of massive and sustained growth of *A. jenneri*, which depletes the nitrogen in the ecosystem.

CHARACTERIZATION OF A NEW PEPTIDE-ALDEHYDE COMPOUND FROM THE TERRESTRIAL CYANOBACTERIUM *NOSTOC* SP. BAHAR_M BY LC-MS AND MARFEY'S ANALYSIS

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Cyanobacteria are a diverse and ancient group of photosynthetic prokaryotic organisms that can inhabit a wide range of environments including extreme conditions such as hot springs, desert soils and the Antarctic. They are abundant producers of natural products recognized for their bioactivity and utility in drug discovery and biotechnology applications. Novel intracellular and extracellular compounds from various cultured and field cyanobacteria with diverse biological activities and a wide range of chemical classes have considerable potential for development of pharmaceuticals and other biomedical applications. However, cyanobacteria are still viewed as an unexplored source of potential drugs. In particular, the collections of cyanobacterial strains from paddy fields of north Iran, where biodiversity is high, are still largely unexplored. We investigated one *Nostoc* strain, isolated from soil samples which were regularly collected from rice fields in the Golestan province of Iran, to search for new compounds. The chemical structure of a newly identified metabolite was elucidated by liquid chromatography-mass spectrophotometer (LC-MS) and Marfey's analysis of the degradation products. We found a new peptide aldehyde compound from a hydrophilic extract of *Nostoc* sp. Bahar_M, which was composed of three subunits, 2-hydroxy-4-(4-hydroxyphenyl) butanoic acid (Hhpba), L-Ile, and L-argininal. According to the structural information, we made the prediction that the new peptide-aldehyde compounds might be a trypsin inhibitor. The presented results proved that soil cyanobacteria are a promising source to yield chemical and pharmaceutical interesting compounds.

IDENTIFICATION OF FOUR DIFFERENT CHLOROPHYLL A ALLOMERS OF *NOSTOC* SP.

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Cyanobacteria (blue green algae) differ from other types of bacteria in that they have chlorophyll a, which other photosynthetic bacteria don't have. In this study, liquid chromatography-mass spectrophotometer (LC-MS) has been used for identification of the four different allomers of chlorophyll a (HO-chl a, MeO- chl a and MeO-lact-chl a), the hydroxy allomer of chlorophyll a (HO-chl a) and the hydroxy and methoxylactone allomers of chlorophyll a (HO- chlorophyll a and MeO-lact- chlorophyll a) from *Nostoc* sp. The differences in mass spectrometric fragmentation of the extracted ion chromatogram can be used as a diagnostic tool for the assignment of the configuration of four different chlorophyll allomers. This case is the first documented of identification of four different chlorophyll a allomers from *Nostoc* sp. in Iran.

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DISTRIBUTION OF SOLIERIACEAE (RHODOPHYTA) IN MEXICO

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Solieriaceae is the most diverse family in the order Gigartinales. Its distributional range includes the tropical and subtropical oceans. The representatives are characterized by filamentous medullary cells, a pseudoparenchymatous cortex, zonate tetrasporangia, and the presence of a cystocarp in which the gonimoblast grows internally from an auxiliary cell. The family is very important economically, because almost all its genera contain carrageenans in their cell walls. Twenty one out of the ninety recognized species have been recorded in Mexico. The objective of this study was to define the distribution patterns of Solieriaceae in Mexico. From the distribution data of the species along the Mexican coast, a PAE analysis (Parsimony of Areas of Endemisms)

was performed to show the distribution areas. Additionally, a phylogenetic analysis was performed in Mr. Bayes, using both our material as well as GenBank *rbcL* and COI sequences, and a cladogram of areas was generated. PAE analysis results shows two large congruence areas: 1) Gulf of Mexico and Mexican Caribbean, including the Yucatan Peninsula and Florida (*Agardhiella*, *Eucheuma*, *Flabaultia*, *Kappaphycus*, *Meristotheca*, *Solieria*, and *Wurdemannia*); and 2) Subtropical Mexican Pacific (*Agardhiella*, *Gardneriella*, *Sarcodiotheca*, *Tacannosca*, and *Wurdemannia*). However, this disjunct generic distribution in Mexico does not imply that they are restricted to either Pacific or Atlantic Oceans. Phylogenetic analysis shows also two major clades: one that shared *Meristotheca* in the Gulf of Mexico and Caribbean, with those species for the Indo-Pacific; and its sister group including *Agardhiella*, *Sarcodiotheca*, and *Tacannosca*, with representatives in the Gulf of Mexico/Mexican Caribbean, and the Indo-Pacific. The vicariant event that we identified as responsible for all the internal dichotomies of the phylogenetic tree was the closure of the Isthmus of Panama (6 millions years ago), suggesting a Tethyan origin of the family, where all the common ancestors were already at this Palaeo-Ocean.

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INVESTIGATION OF EXPRESSION OF MEDIUM DEPENDENCY OF EYE2 PROTEIN IN CHLAMYDOMONAS REINHARDTII

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Chlamydomonas reinhardtii, a single-celled green alga, has special systems used for detecting incoming light and determining the direction of swimming according to its intensity. These systems are called the "eyespot". Previous research has documented five different proteins that are localized in eyespots and are responsible for both the granulation and the functioning of the eyespot. Also documented are effects on protein expression of different media. The objective of this study was to understand these effects by investigating the change in EYE2 protein expression levels with regard to differences in medium. In this study, wild type *C. reinhardtii* cells were grown in three different

media and the EYE2 protein expression, tested by Western blot, was compared. It has been shown that there are some changes in the level of EYE2 protein expression, depending on the medium.

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A HISTORICAL REVIEW ON THE STUDIES OF MICROPHYTOBENTHOS IN AND AROUND THE KOREAN TIDAL FLATS

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In the present study a comprehensive review of microphytobenthos (MPBs) studies in and around the Korean tidal flats has been done. Particular emphasis was put on recent findings on the ecology and taxonomy of marine benthic diatoms. Assemblages, dynamics, production, and food web etc. have been found to be internationally recognized topics relating to the tidal flat MPBs in chronological order, accordingly a review on the Korean MPBs studies was provided in the given topics. In particular, a brief summary from several representative works related to the corresponding topics was provided for comparison, where applicable. While an approximately 50 year scientific gap between Korea and the European countries was clearly identified, rapid scientific advances in the most recent 10 years are noteworthy. The classical topic of diatom assemblages has been a steady issue in Korea, with documented MPBs of >400 species (ca. 10 new species) from Korean tidal flats. As part of our review, selected data from previous and current works on MPBs encompassing the four topics mentioned above have been reanalyzed. In conclusion, future MPBs studies in Korea should contribute to the proper appreciation of biodiversity and ecological function of marine benthic diatoms in and around Korean tidal flats.

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CYANOBACTERIA-SPECIFIC ALGICIDAL MECHANISM OF THE ECO-FRIENDLY NAPHTHOQUINONE DERIVATIVE (NQ 2-0)

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A harmful-cyanobacteria-specific algicide, naphthoquinone derivatives 2-0 (NQ 2-0) has been developed, however its algicidal mechanism has not been understood. We examined the specific algicidal mechanism of NQ 2-0 using *Microcystis aeruginosa* (cyanobacteria), *Selenastrum capricornutum* (green algae) and *Cyclotella* sp. (diatoms) clonal cultures. Algicidal effects on *M. aeruginosa* were 97.4% and 19.0% under light and dark conditions, respectively, whilst it had no effect on *S. capricornutum* and *Cyclotella* sp. in both conditions. We measured oxygen evolution rate, chlorophyll (Chl) fluorescence and photosynthetic efficiency (*Fv/Fm*) to investigate the effects of NQ 2-0 on photosynthesis. In the case of *M. aeruginosa*, oxygen evolution rate was reduced by 0.6–1.5 times, the Chl fluorescence was higher 11–29% and the *Fv/Fm* was lower 13–22% than the control. In case of *Cyclotella* sp., although oxygen evolution rate was reduced by 0.3–1.0 times compared to the control, it still showed a positive value, and there was no effect on Chl fluorescence and *Fv/Fm* after NQ 2-0 treatment. *S. capricornutum* was not affected by NQ 2-0. Reactive oxygen species (ROS) generation, antioxidant enzyme activities and lipid peroxidation were also measured to evaluate the stress response on photosynthesis. The ROS, lipid peroxidation and antioxidant enzymes activities were significantly increased in *M. aeruginosa* after NQ 2-0 treatment, whilst both *S. capricornutum* and *Cyclotella* sp. were not affected. Additionally, LM and TEM images were taken to observe the morphological changes after NQ 2-0 treatment. The thylakoid membrane of *M. aeruginosa* was degraded at 12 hours and cytoplasmic vacuolation was observed at 24 hours after NQ 2-0 treatment, whilst there were no morphological changes in the other two species. Our results suggest that NQ 2-0 inhibits the photosynthesis of cyanobacteria, increases ROS, lipid peroxidation and destroys the intracellular structure.

COMPARATIVE ANALYSIS OF WHOLE TRANSCRIPTOMES OF PYROPIA YEZOENSIS

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In this study, we carried out a comparative analysis of whole transcriptomes of a wildtype (PyWT) and a high-temperature-tolerant strain (Py500G) of *P. yezoensis* using Next generation RNA Sequencing (RNA-Seq). By sequencing with Illumina HiSeq 2000 system, 184,381,412 paired-end reads with the length of 151 nt were obtained. De novo assembly generated 31,147 and 78,793 unigenes for Py500G and PyWT, respectively. To facilitate downstream analysis, the two transcriptomes were annotated by searching homologous sequences in NCBI NR, Swissprot, Pfam, and KEGG databases. To unravel the differences in Py500G and PyWT, we first identified the shared sequences of Py500G and PyWT. Next, we analyzed expression patterns and identified differentially expressed unigenes. GO terms retrieved via InterProScan were used to perform GO enrichment analysis of DEG list. All the analyzed data were finally integrated to form a candidate gene list that might be responsible for Py500G phenotype. There were 144 candidate genes proposed to be important in explaining the difference between Py500G and PyWT, including elongation factor 1A gene, proton-pumping pyrophosphate, and two genes of the oxidative phase of the pentose phosphate pathway – glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase. This study not only offers the candidate genes responsible for the difference in the heat-tolerant strain Py500G and PyWT at the molecular basis but also provides high quality genomic data for future genetic research on *P. yezoensis*.

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PLASTID & MITOCHONDRIAL GENOMES OF MINERVA AENIGMATA, AN EARLY DIVERGED SPECIES OF BANGIALES (RHODOPHYTA)

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Minerva aenigmata is a species in the Bangiales with unbranched, erect thalli, initially forming uniseriate filaments that become multiseriate at maturity. This simple filamentous structure is morphologically indistinguishable from other filamentous Bangiales taxa. *M. aenigmata* is known as the earliest-diverging species of Bangiales and is distributed in the upper intertidal zone along the New Zealand coast. Currently, one *Bangia*, two *Porphyra*, and six *Pyropia* plastid genomes are available, however, these taxa all belong to the stem group of the Bangiales. To better understand organelle genome evolution, we sequenced and annotated the complete plastid and mitochondrial genomes of the early-diverged *M. aenigmata*. The plastid genome length is 189.5 kbp with 32.3% GC content. The circular plastid genome contains 247 genes including 203 protein-coding genes, 6 rRNAs and 38 tRNAs. The mitochondrial genome is 29.7 kbp in size with 30.8% of GC that encoded 21 protein-coding genes. Comparative analyses of organelle genomes show highly conserved genome structures including genome sizes, gene contents, gene orders and intron regions both in plastid and mitochondrial genomes. In this presentation, we will discuss possible evolutionary scenarios for the high conservation of organelle genomes of the Bangiophyceae.

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PELAGIBACA BERMUDENSIS PROMOTES BIOFUEL POTENTIAL OF TETRASELMIS STRIATA IN BROAD RANGE OF STRESSORS IN ADDITION TO THE RELEASE OF HHQ AND PQS

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The marine bacterium *Pelagibaca bermudensis* and the potential biofuel algal strain *Tetraselmis striata* were

co-cultivated under varying pH, salinity, temperature and light conditions, and compared with the *Tetraselmis striata* cultivated under axenic conditions. In all of the experimental sets, biomass and lipid production in the co-cultivated conditions were higher than the axenic conditions. There was multi-fold increase in growth promotion effect, with higher cell abundance under optimal conditions in presence of *P. bermudensis*, with ameliorated lipid productivity. An independent investigation of the effect of crude extracts containing metabolite and nutrients released by *P. bermudensis* on the microalgal cells also exhibited elevated biomass and higher growth. However, *P. bermudensis* leads to slight reduction of lipid content of biomass under certain conditions, but provides clues for the enhanced survival of microalgal cells in presence of the *P. bermudensis* and released metabolites in broad spectrum of environmental stressors. This co-cultivation strategy can be used for the amelioration of *T. striata* biomass production in saline effluents/ seawater based-media with broad pH ranges and with varying temperature/light and salinity conditions. We also studied the bacterial growth dynamics in the presence of this microalga and results showed unique growth trends in each case. The bacteria were found to release quorum sensing precursors HHQ and PQS in most of the varying environmental conditions which did not hamper the growth promoting effect on the microalgae and could lead to a typical growth curve for the survival. The majority of quorum sensing lactones were found to be absent but quinolones were present. *P. bermudensis* could not grow in the absence of *T. striata* in microalgal growth media (O3).

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CYANOBACTERIA AS A FACTOR DIFFERENTIATING BETWEEN SMALL AND LARGE CHARA-LAKES

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There is still little known about cyanobacteria in oligo- and mesotrophic hard-water lakes dominated by charophyte vegetation (Chara-lakes). In such ecosystems, through various mechanisms (e.g. nutrient trapping, intensified sedimentation of particles from the water column and discharge of allelopathic substances), charophytes affect

the qualitative and quantitative structure of phytoplankton. Even though anthropogenic pressure on a lake and its catchment area may bring a risk of cyanobacteria blooms. Therefore, the aim of this study was to find out whether there are any differences in the qualitative and quantitative structure of cyanobacteria and in cyanobacteria functional groups between Chara-lakes which vary in terms of morphology and human impact. Two small, mid-forest Chara-lakes were compared with two large, recreationally used Chara-lakes placed in largely afforested area. In each lake the qualitative and quantitative structure of cyanobacteria was analyzed for three sites within the pelagic zone. Additionally, physical-chemical analyses of the surface waters were performed.

The results showed that Chroococcales taxa distinguished small mid-forest Chara-lakes, while Oscillatoriales and Nostocales preferred large recreationally used water bodies. The abundance of *Aphanothece minutissima*, *Merismopedia tenuissima*, *Aphanizomenon gracile*, *Aphanocapsa holsatica* and *Cyanodictyon planctonicum* varied most significantly among the studied Chara-lakes.

The pattern of cyanobacteria dynamics did not distinguish the Chara-lakes from eutrophic water bodies with phytoplankton dominance. However, only in the two large Chara-lakes, characterized by a worse ecological status than the small mid-forest ones, was the dominance of cyanobacteria detected. Apart from the known impact of trophy and in addition to the abundant underwater charophyte meadows, water alkalinity, colour, and mixing regime seem to play key roles in the development of cyanobacteria in Chara-lakes. The lake morphology and its recreational use, along with a drainage basin subject to human activity, enhance the risk of an increased proportion of cyanobacteria in the phytoplankton community of Chara-lakes.

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CORALLINE RED ALGAE: NEW INSIGHTS CHALLENGE OUR UNDERSTANDING OF A GLOBAL BENTHIC GROUP

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Coralline red algae have a global distribution, and a high ecological relevance as they form bioconstructions and therefore function as engineer species. Furthermore, corallines are extremely diverse by themselves and they create entangled 3D hard structures which serve as habitat for many organisms. They are considered as a marine group, except for the recent discovery of a genuine freshwater

species (*Pneophyllum cetinaensis*). If the affinity of the coralline initially puzzled the first naturalists who study them, Philippi (1837) recognized them as photosynthetic organisms. Since then, their taxonomy and systematics has been influenced by different methodological approaches. For instance, the literature shows an early prolific period of species descriptions, mostly based on gross morphology as the anatomical study of this calcareous organisms was difficult. Recently, the application of molecular phylogenies fostered our understanding of their affinities within the Florideophyceae. Likewise, this approach allowed us to detect distinctive structural and reproductive characters pertaining to the three phases of their life cycle which show evolutionary trends along the different coralline lineages. As calcareous organisms, coralline algae left an extensive fossil record which was used as a timeline for calibrating phylogenies and inferring the evolutionary novelties. Moreover, our assessments of species diversity using DNA barcoding approaches revealed a tremendous species diversity with many cryptic species. The sequencing of type specimens, which can be achieved with a relatively high success for the corallines, allowed us to bridge the gap between operation taxonomic unit and taxonomic names. Nevertheless, while new taxa continue to be discovered, our research on coralline algae faces more than 1600 species and infraspecific taxa described, many of them still unchecked and unsequenced.

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**LIVING AETHESOLITHON J.H. JOHNSON
(CORALLINALES, RHODOPHYTA):
NEW INSIGHTS ON ITS PHYLOGENETIC
POSITION USING MOLECULAR
AND MORPHO-ANATOMICAL DATA**

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The genus *Aethesolithon* was established by Johnson in 1964 based on Miocene fossils of coralline algae from Guam, Mariana Islands. The type species, *A. problematicum* J.H. Johnson, was described in the Bonya Limestone, dated as Tertiary f, which now is known to extend from the early Miocene (Burdigalian) to the late Miocene. According to the protologue, *Aethesolithon* was separated from

other genera by its large polygonal cells. The type species is characterized by “a single layer of horizontally elongated and slightly oblique basal cells” and “the rest of the crust formed of very irregular layers or lenticular masses of polygonal cells”. Conceptacles occurring in the syntypes are small (140–200 µm in diameter and 100–140 µm high) and were interpreted as uniporate. In the same paper, the author described another species in the genus, *A. grandis* J.H. Johnson, characterized by larger cells and conceptacles, and from younger rocks (late Miocene to Pliocene) from the island. A third, early-Miocene *Aethesolithon* species, *A. guatemalaensum* J.H. Johnson & Kaska was described from Guatemala and separated from the two former species by its smaller cells. Fossil *Aethesolithon* have been found in Miocene to Holocene rocks in many localities in the Indo-Pacific. However, thus far no living corallines with the characteristic vegetative anatomy of the genus have been described. Recently, a collection identified as an *Aethesolithon* species was found alive in Lizard Island, in the northern Great Barrier Reef. Molecular data revealed the phylogenetic position of *Aethesolithon* within the subfamily Hydrolithoideae, conspecific with further collections from the south Pacific islands. Although our specimen was vegetative, examination of the other collections revealed that sporangial conceptacles are uniporate.

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**PRODUCTIVITY OF MACROCYSTIS
PYRIFERA JUVENILE UNDER CONTROLLED
CONDITIONS: WHY MORPHOLOGY
MATTERS?**

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By producing female gametophytes with few (≤ 2) or multiple oogonia (≥ 3) it is possible to produce juvenile sporophytes with few or multiple fronds. In this study, we tested whether productivity of juvenile kelp can be enhanced by manipulating its morphology and using controlled experimental conditions. *M. pyrifera* reproductive material was

collected in southern Chile (41°37'S; 73°04'W) and cultivated under standardized culture conditions to obtain a new generation of sporophytes (1–2 cm in length). Two different factorial experiments with juvenile kelp sporophytes (2–5 cm) showing low and multiple number of fronds were performed: combination of different temperatures (12° and 18°C) and light regimes (20 and 45 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$), keeping the nutrient concentration according to Provasoli medium. Growth was assessed by measuring the individual biomass and photosynthetic area, and the physiological and productivity performance by evaluating photosynthetic parameters (i.e. pigment concentration, quantum efficiency, electron transport rate and the saturating irradiance for photosynthesis using a pulse amplitude fluorometer (PAM). The results indicate that few-frond juvenile sporophytes present a significantly higher individual blade increment, both in length and width. However, as the kelp individual presents a larger number of blades, the total biomass and total photosynthetic surface was significantly higher in the multiple-frond *M. pyrifera* individuals. PAM measurements reveal that photosynthetic capacity does not show significant differences when morphologies are compared. However, the productivity reached an average value of $27.63 \pm 6.15 \mu\text{mol C s}^{-1}$ per kelp individual in multiple fronds *M. pyrifera* and was significantly lower in few fronds individuals ($17.61 \pm 1.08 \mu\text{mol C s}^{-1}$ per kelp individual). We discuss these results within a life history-adaptative context.

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INTERACTIONS BETWEEN ENGINEERED MICROBIOMES AND NATURAL BACTERIAL COMMUNITIES

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Engineered microbiomes, mainly bacteria and fungi, have been used since decades in numerous fields including medicine, industry and agriculture. For the purpose of phytoprotection or bioremediation, engineered microbiomes – genetically modified or not – are thus released into the environment. Before any release in an uncontained environment, the impacts of engineered organisms on the natural microbial communities need to be considered. Predictive ecology studies help evaluate these potential impacts and to balance their expected benefits against any potential risks.

A solid knowledgebase acquired on microbiomes engineered for land-based agriculture shows that the evaluation of ecological risks is complex and should be based on multiple criteria. This complexity will be illustrated through the example of the soil-dwelling, nitrogen-fixating bacteria *Bradyrhizobium*, intentionally introduced for *Acacia* agroforestry. Several studies have been conducted on different temporal and spatial scales, starting from short-term laboratory experiments, down to greenhouses and small-scale field trials in an open environment. Finally, long-term monitoring was performed in very different ecosystems, at a worldwide scale. These case studies help to measure the persistence of introduced organisms, their competition with native species and their subsequent impact on the local microflora, as well as the potential genetic exchange between introduced and native microbiome.

There is growing interest to engineer algae or their microbiomes for industrial applications such as biofuel production or the degradation of organic pollutants raises questions on their interactions with natural communities; this is compounded by the development of efficient technologies towards the genetic modification of algae and their microbiome. As in agriculture, and parallel to the current aquaculture boom, the scientific community will have to review, evaluate and monitor the ecological risks associated to engineer algae and their microbiomes.

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ZYGNEMA SP. FROM POLAR AND ALPINE TERRESTRIAL HABITATS: ADAPTATIONS TO SIMULATED UV RADIATION INVESTIGATED BY A PHYSIOLOGICAL AND METABOLOMICS APPROACH

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The streptophytic green algae of the genus *Zygnema* form conspicuous mats in polar and alpine terrestrial habitats where they are exposed to environmental stresses including

UV radiation. We investigated three isolates (Arctic, Antarctic and Alpine) for their tolerance to experimental UV treatment. We exposed samples of young vegetative cells (one month old) and pre-akinetes (6 months old) to experimental sun simulation with an elevated, but realistic UV scenario. Measurements of the rETR rate showed a decrease in vegetative cells of two strains. The effective quantum yields, generally lower in pre-akinetes, were not drastically changed during the UV treatment. Transmission electron microscopy of young cells showed a tentative increase in electron dense bodies in UV AB treated cells, likely indicating UV protecting components. Chromatographic analysis (HPLC) allowed the detection of two phenolic compounds with UV absorbing capacities that drastically increase in young cells after UVR treatment. Analysis of the primary pigment composition revealed a decrease in chlorophyll a after UV exposure and an activation of the xanthophyll cycle pigments. Metabolite profiling using non-targeted RP-UPLC-MS allowed a clear separation of the strains and a clear distinction between old and young cells. However, no correlation to the treatments was observed. This suggests that only particular components, like the phenolic compounds and pigments are changed, providing *Zygnema* sp. with suitable protection against the applied UV stress. These observations explain the high UV tolerance of terrestrial *Zygnema* strains.

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PHOTOSYNTHETIC ADAPTATIONS
TO TERRESTRIAL ENVIRONMENTS
IN EARLY AND LATE BRANCHING
STREPTOPHYTE GREEN ALGAE
KLEBSORMIDIUM AND ZYGNEMA

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Streptophyte green algae (SGA) are a highly diverse group comprising many species adapted to aero- terrestrial habitats. Among them, *Klebsormidium* and *Zygnema* are representative members of the two recently designed evolutionary clades, the basal and advanced branching SGA, respectively. Currently, the Zygnematophyceae are considered as sister group to land plants. In this study, we aim to understand the physiological traits of both genera in relation to ecological parameters (light, water and CO₂) which have driven their adaptations to land. We worked on a newly isolated *Klebsormidium* sp. from an acidic habitat,

in comparison to an alpine *K. flaccidum* isolate, and a *Zygnema* sp. collected from a sandy littoral river. The results showed that *Klebsormidium* had a higher and inducible photoprotective (NPQ) capacity than *Zygnema*, making it tolerant to high light intensity. However, photoinhibition occurred with rapid increases of light, suggesting that NPQ in *Klebsormidium* is most efficient against intense and stable light conditions. The photosynthetic apparatus of *Klebsormidium* also showed resistance to dehydration in low light.

However, when dehydration took place in a relatively high light environment (185 μmol photons m⁻² s⁻¹), a harmful over-reduction of the electron transport chain occurred, which impaired the ability to recover after rehydration. We suggest that exposure to dehydration in nature is an important force shaping the species adaptation towards low light. The ability of SGA to live on land depends also on their capacity to acquire inorganic carbon (Ci). Due to the higher restriction of *Zygnema* to water environments, we hypothesise that *Zygnema* might have a better ability to use HCO₃⁻ than *Klebsormidium*. Contrary to this expectation, our preliminary results indicated that both *Klebsormidium* and *Zygnema* equally and predominantly rely upon CO₂ (more than HCO₃⁻) as Ci source, suggesting a similar adaptation of their CO₂-concentrating mechanisms.

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OPENING PANDORA'S BOX: A DIATOM
SPECIES COMPLEX AS A CASE STUDY
FOR THE DIVERSITY AND BIOGEOGRAPHY
OF TERRESTRIAL MICRO-EUKARYOTES

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Because of their immense diversity, the systematics of protists, and in particular of diatoms, is complex and challenging. As species taxonomy has far-reaching consequences for our understanding of diatom evolutionary history, ecology and biogeography, detailed studies into species limits and diversity are needed if we are to advance our understanding of the range dynamics and diversification of diatoms. However, the biogeography and ecology of (pseudo)cryptic diatom species is highly understudied and there are virtually no data available on the timing of lineage splitting within species complexes. To tackle these issues, we used the terrestrial diatom complex *Pinnularia borealis* – *Pinnularia rabenhorstii* as a case study for diatom species complexes, and more generally microscopic eukaryotes. Sample collection on multiple continents, including Antarctica, resulted in the establishment of over 1,000 monoclonal strains. Automatic species delimitation based on the *cox1* and *28S* genes revealed the presence of at least 70 lineages worldwide and species accumulation curves indicated that the true global diversity is likely to exceed several hundreds of lineages. Despite the high genetic diversity, morphological divergence is limited. A molecular clock analysis on a four-marker phylogeny indicated that the diversification likely started in the late Eocene and strongly increased during the Miocene. Whereas some lineages are cosmopolitan, most seem to have restricted geographic distributions. The results indicate a high degree of hidden species diversity worldwide and the presence of regional endemics in Antarctica. The topology of the phylogenetic tree and the age of the lineage splitting suggest multiple independent colonization events in or out all continents during the past 20 million years. Together with the high levels of genetic diversity, this suggests that although this diatom complex is relatively successful in dispersal, dispersal is not sufficiently frequent to prevent speciation. Altogether, these findings provide evidence for the moderate endemism hypothesis for micro-organisms.

MIOCENE DIATOM FOSSILS SHED LIGHT ON THE PALEOENVIRONMENTAL AND BIOGEOGRAPHICAL HISTORY OF THE ANTARCTIC CONTINENT

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In contrast to the rich marine fossil record that extends to the late Mesozoic, records of pre-Quaternary lacustrine diatom deposits are relatively scarce. Such records provide information concerning paleoenvironmental change, as well as new insights concerning the evolution and biogeography of freshwater diatom floras. Here, we report two well-preserved lacustrine diatom assemblages from the Transantarctic Mountains in Continental Antarctica dating back to the Middle Miocene (ca. 14–17.5 Ma): Mount Boreas in the Olympus Range in the western Dry Valleys, and the Friis Hills adjacent to the Asgard Range in the southern Dry Valleys. Detailed diatom counts of the Mount Boreas sediments suggest that the Mount Boreas lake persisted for several thousands of years and underwent progressive natural acidification. Interestingly, the analysis of both fossil assemblages indicates that a rich diatom flora existed in Continental Antarctica, with marked Gondwanan flag ship genera providing evidence that, also on the microorganism level, Antarctica showed affinity with the Gondwanan continents. The Miocene material shared many genera with modern alpine and Arctic lakes, but at the morphospecies

level, the overwhelming majority of the Miocene taxa could not be assigned to modern species, although many of the known species complexes/groups dominating contemporary Antarctic floras were already present. In contrast, since the early Holocene, a modern continental diatom flora is present, being severely impoverished and strongly enriched in (semi-)terrestrial taxa, suggesting that the Mid Miocene cooling event (ca. 14 Ma) and the subsequent expansion of ice sheets, resulted in selective extinction and the evolution of a species-poor yet highly adapted and largely endemic modern diatom flora. Together, these new fossil diatom localities shed a unique light on the evolution of Antarctic lake ecosystems and taxonomic and biogeographic aspects of the Antarctic freshwater flora.

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MANIPULATING ULVA-ASSOCIATED BACTERIAL COMMUNITIES: A POSSIBLE STRATEGY FOR BACTERIAL CONTROL IN IMTA-RAS CULTURES?

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This work was conducted in the frame of the IntegRAS project (AGL2013-41868-R), funded by the Spanish government, with the overall objective of optimizing the design and management of a sole-*Ulva* culture in an Integrated Multitrophic Recirculating Aquaculture System (IMTA-RAS), minimizing energy consumption, seawater renovation rate and improving the productivity and health of the system. *Ulva* spp., provide an important niche for biofilm-forming bacteria, including those from the *Phaeobacter* genus that can reduce growth and kill fish pathogens. These antibiotic-producing bacteria (APB), grown on biofilters, have demonstrated their effectiveness as probiotic

in aquaculture by reducing the mortality of vibrio-challenged fish larvae. As epibiotic communities in macroalgae may be randomly formed by functionally equivalent guild members present in the surrounding seawater, we hypothesize that experimental colonization of *Ulva* spp. with selected algal-epiphytic APB could control pathogenic or opportunistic bacteria in IMTA-RAS systems, improving fish health. Samples of *Ulva australis* and *U. rigida*, sharing the same intertidal area, taken in Galicia (NW Spain) showed that bacterial communities on *Ulva* species were distinct from planktonic bacteria and showed host specificity. Both *Ulva* species hosted *Phaeobacter* strains with antagonism against *Vibrio anguillarum*. The experimental colonization and the detachment kinetics of those bacteria on the thallus of different *Ulva* species was studied using a multi-well-based testing system and the effect of the introduced bacteria on the growth and microbial assemblages of the algae were analyzed. Based on those results, a co-culture of the selected species of *Ulva* and *Phaeobacter* was scaled-up and the maintenance of the introduced *Phaeobacter* was monitored. The antagonistic activity of the *Phaeobacter*-colonised *Ulva* against a fish-pathogenic *V. anguillarum* strain was analysed by *in vitro* and *in vivo* trials.

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EFFECTS OF TEMPERATURE AND NUTRIENTS ON GROWTH, SURVIVAL AND PHYSIOLOGY OF SIX INTERTIDAL SEAWEEDS

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There is evidence that some seaweeds have changed in abundance and range along the North Atlantic in recent years, and climatic drivers are often invoked as potential culprits for these changes. In the particular case of north-west Iberia, recent decadal changes in the frequency of occurrence of several intertidal perennial seaweeds coincided with variations in temperature and nutrient regimes. To further investigate whether water warming and nutrient

availability may be behind the observed changes, the influence of temperature (14°, 18°, 22°, and 26°C) and nutrient availability (high vs low) on growth, survival and physiology was compared among six intertidal seaweeds whose frequency has recently changed in NW Iberia. The experiment design involved weekly (growth, survival) and final (photosynthesis, respiration, quantum yield) recordings over a seven weeks exposure. By comparing the responses of species that recently followed diverging upward and downward trends, we will be able to better assess the actual role played by temperature and nutrient availability as drivers of the trends observed in the field.

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PHOTOSYNTHESIS SENSITIVITY TO TEMPERATURE OF EIGHT INTERTIDAL SEAWEEDES MATCHES RECENT DEMOGRAPHIC TRENDS

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Macroalgae are important providers of habitat and food to many organism. In recent years, changes in the abundance and distribution of some seaweeds have been detected all over the world. These changes have been mainly attributed to global warming. In NW Iberia, decadal changes in the frequency of occurrence of several intertidal perennial seaweeds seemingly occurred in parallel with an increase of sea water temperature, particularly in autumn. To better understand the actual role played by temperature in these recent shifts, we compared the response to temperature of eight intertidal seaweeds that recently followed contrasting upward and downward frequency changes in NW Iberia. Optimum temperature (T_{opt}) and temperature sensitivity were assessed for photosynthesis and respiration in short-term exposures under controlled laboratory conditions. T_{opt} estimates varied significantly among some seaweeds but no consistent pattern could be detected between downward and upward algae. Likewise, maximum rate of primary productivity (gross and net) at T_{opt} also differed significantly among some species but these differences seemed unrelated to whether the seaweeds belonged to the upward or downward set. In comparison, the temperature dependency of photosynthesis (rate of change as a consequence of

increasing temperature) was consistently and significantly higher in upward than in downward algae, indicating that productivity was more responsive to temperature in the former. The implications of this observation for the potential role of temperature in the recent diverging trends detected in field surveys are discussed.

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ENVIRONMENTAL AND SPATIAL INFLUENCES ON BIOGEOGRAPHY AND COMMUNITY STRUCTURE OF BENTHIC DIATOMS

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Several theoretical and practical reasons suggest that benthic microalgae could be useful bioindicators. For instance, an ideal indicator species or community would be associated with a given habitat due to local physical conditions or biotic interactions (i.e., ‘environmental filtering’), not due to dispersal limitation. Due to their small size, immense abundances, and reliance on passive dispersal, the popular notion about micro-organisms is that ‘Everything is everywhere, but, the environment selects’ (Baas-Becking 1934). Although much recent research concerning planktonic bacteria and dispersal limitation has been conducted, little in this regard is known about microeukaryotes, especially benthic microbes. The purpose of our study was to identify and compare spatial and environmental influences on benthic diatom community structure and biogeography. In summer 2015, sediment was sampled at various spatial scales from four barrier island beaches in South Carolina, USA, and high-throughput DNA sequencing was used to characterize diatom assemblages. Analysis of Similarity (ANOSIM) and principal coordinates analysis revealed that communities were statistically distinct on the four islands. Community dissimilarity was compared to both spatial distance and environmental differences to determine potential influences of these variables on community structure. At large spatial scales (10–100 km), we found that geographic distance had the strongest correlation with community similarity, while differences in temperature (air and sediment), sediment organic matter, and nutrients also had significant but weaker relationships with community structure. Surprisingly, temperature, which changes on very short time scales, appeared to be a dominant factor related to diatom species composition, potentially

implicating some unmeasured variable (e.g., cloud cover). At small scales (m – hm) little spatial structure was observed, although community dissimilarity did correlate with organic matter difference on one barrier island. Future research will explore how experimental and natural changes in light and temperature alter diatom community structure over short temporal scales.

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CHEMICAL COMMUNICATION IN MICROALGAE – HOW UNICELLULAR ORGANISMS SHAPE AND PERCEIVE THEIR ENVIRONMENT

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Unicellular algae in the plankton and in biofilms have established means to interact with other organisms in their environment. Especially interactions mediated by chemical compounds have gained a lot of attention during the last years. Algal exudates as well as metabolites stored in the cells can mediate feeding activity of herbivores and algal / algal interactions but also communication of an alga with the surrounding microbial community. We introduce an approach to address such chemically mediated interactions by combining ecological and analytical investigations of microalgae in stress and interaction situations. These surveys reveal that algae exhibit a high plasticity of their metabolome as an adaptation to varying biotic and abiotic environmental factors. Bioassays that are guided by metabolomic evidence reveal that these specific compounds can mediate mate finding, chemical defense or algal / algal interactions in a highly dynamic manner. Examples illustrating this complex interplay will be introduced and consequences for future investigations of plankton ecology, algal physiology, and chemical interactions are discussed.

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STUDY ON SIMILARITIES AND DIFFERENCES OF THE SPECIES TRACHELOMONAS VOLZII AND T. DUBIA (EUGLENOPHYCEAE) – ONE OR TWO INDEPENDENT SPECIES?

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Two species *Trachelomonas volzii* and *T. dubia* were examined for similarities and dissimilarities of their loricae and monads organization. We specifically focused on the key features of both species that were originally used to separate one from the other: annular thickening at the base of the collar and dimensions and ornamentation of loricae. Loricae of specimens were examined by light and scanning electron microscopy and the results were compared with the literature data describing these taxa and reported occurrences throughout the world. The species together with their varieties and forms do not appear to have strong characteristics distinguishing them, rather, the variability observed fits natural phenotypic changes. Based on evidence from this study, we recommend combining these two taxa and propose *T. volzii* as the singular taxon. We also examined a set of varieties of original *T. volzii*, since the species contained several varieties that were almost identical in relation to lorica structure and occurrence. As a result of these observations, we propose the following varieties: *Trachelomonas volzii* var. *volzii* as a nominative variety, *T. volzii* var. *australis*, *T. volzii* var. *sulcata*, *T. volzii* var. *inflata*, *T. volzii* var. *acidophila*. Furthermore, we propose renaming *T. dubia* var. *ornata* to *T. volzii* var. *ornata* and *T. dubia* var. *colliundulata* to *T. volzii* var. *colliundulata*. In our opinion, *T. dubia* fo. *acuminata* should be included with the species *T. hexangulata* due to its unique, hexagonal shaped lorica.

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DIVERSITY OF EPILITHIC AND ENDOLITHIC CYANOBACTERIA AND GREEN ALGAE AT THE ENTRANCE OF TWO CAVES IN SERBIA

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Epilithic and endolithic cyanobacteria and green algae were investigated from carbonate stone substrata with visible developed biofilm at the entrance of two caves in Serbia: Petnička cave and Cerjanska cave. The epilithic community analysis revealed the presence of 43 cyanobacterial taxa and five taxa belonging to Chlorophyta. When observing cyanobacteria, coccoid forms were the most diverse (31 taxa in total, of which 28 were found only in Cerjanska cave), with representatives from the genera *Gloeocapsa*, *Chroococcus*, *Aphanocapsa*, *Aphanothece* and *Gloethece* as the most frequently encountered. Representatives of *Leptolyngbya* and *Phormidium* were the most common filamentous cyanobacteria in both caves, while heterocytous taxa, mostly belonging to the genus *Nostoc*, were recorded only in Cerjanska cave. Chlorophyta were mostly found in Cerjanska cave, with *Desmococcus*, *Trochiscia* and *Klebsormidium* being dominant. The presence of taxa capable of precipitating CaCO₃ (a common phenomenon in aerophytic cyanobacteria) was documented in both caves and was observed using Scanning Electron Microscopy. For the isolation of endolithic cyanobacteria and algae from the carbonate rock substratum, a technique that involves hydrochloric acid was used. The chosen hydrochloric acid concentration of 10% was strong enough to dissolve the surrounding matrix, leaving cyanobacterial and algal cells and their sheaths undamaged. An interesting endolithic *Leptolyngbya*-like cyanobacterium was found in Petnička cave, characterized by: presence of olive green trichomes constricted at cross-walls, cells that are slightly longer than wide (1.71–2.86 × 1.55–1.79 μm) and a well developed colourless to light brown sheath. Scanning Electron Microscopy revealed a great potential for this apparently actively boring cyanobacterium (possible euendolith) in the deterioration of carbonate substrata. Floristic composition, recorded in both caves, indicates the presence of specific assemblages that unify aerophytic cyanobacteria and algae. Diversity studies of epilithic and endolithic phototrophs

represent a solid base for better understanding of pioneer species and their strategies.

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EUROPEAN PERSPECTIVES OF REGULATION FOR SEAWEED BREEDING AND SEEDLING PRODUCTION IN A GENOMICS ERA

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In comparison to the extensive seaweed farms of Asia, seaweed aquaculture in Europe is very small scale. The main activity of the European seaweed industry involves harvesting and exploiting natural populations mainly for the colloid and plant biostimulants industry. Developing concern about environmental impacts due to harvesting pressure on wild populations has encouraged seaweed farming projects throughout European waters. Expansion of industries based on macroalgae can only be practicably achieved through algal farming.

From a biological point of view, the potential impact on seaweed genetic diversity has triggered concern for sustainable practices. Preserving natural genetic diversity is a pre-requisite for developing efficient resource production through farming. Compared to cultivars selected for land agriculture (maize, wheat...), strain selection for seaweed farming is at a very early age and an inevitable leap in knowledge is expected. EU countries are facing common technological challenges, environmental concerns as well as financial and legal barriers.

For further optimisation of seaweed mariculture systems, more focus is required on the production of species for high-value markets and of better adapted varieties selected from local populations. As marine environments are less controllable and are more variable than land-based systems, breeding should aim at improved strains adapted to the local environment as well as preventing the introgression of unsuitable genes in natural populations. This talk will discuss some important questions that arise with respect to our efficiency in selecting the most important traits for the brown algal kelp *Saccharina latissima*, such as yield stability, and on the compounds and substances to

produce and apply them in a the context of the preservation of natural populations.. Perspectives for a global regulation for seaweed breeding and seed production in EU will be given that could also be implemented in other regions of the world.

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EVOLUTIONARY RELATIONSHIPS OF THE RED ALGAL PARASITE PTEROCLADIOPHILA HEMISPHAERICA USING COMPLETE MITOCHONDRIAL AND 'GHOST' PLASTID GENOMES

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Red algal parasites grow on other red algae with 120 taxonomically recognised species world-wide. Most are taxonomically closely related to their hosts. This unique group is poorly studied and only two mitochondrial and one plastid genomes have been sequenced in red algal parasites. Based on this limited data it seems that the mitochondria is similar to free-living red algal mitochondria in gene content, while the plastid is highly reduced with only one photosynthetic gene remaining ('ghost plastid'). Currently 10 red algal parasites are found in New Zealand. One of these is *Pterocladiphila hemisphaerica*, taxonomically placed in the order Gracilariales but growing on the host *Pterocladia lucida* in the Gelidiales. Next – generation sequencing was used to sequence organelle genomes in *Pterocladiphila hemisphaerica* and its host. Complete mitochondrial genomes in the host and parasite are quite similar in genome size, gene order and gene size. Mitochondrial protein coding genes were used to investigate phylogenetic relationships of the parasite to its host and representative in the Gracilariales. The ghost plastid genome in the parasite is only 70 k bp with only one photosynthesis gene whereas the host plastid is over 175 k bp long. Preliminary data suggests parasite organelles show different histories, and the origin of the parasite is neither in the family Gracilariales (current taxonomic placement) nor the host family (Gelidiales). Our data will add to our limited knowledge of parasites and their origins and this is the first genomic study of red algal parasites in New Zealand.

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TERRESTRIAL VS MARINE AGRONOMY: TIMELINES AND LESSONS TO BE LEARNED

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The estimated value of the seaweed industry was US\$10.1-16.1 billion in 2015, with some reports suggesting c. US\$17.6 billion by 2021. The total annual, global seaweed production in 2014 was 28.5 million tons; cultivation accounting for 96% (i.e. 1.2 million tons wild harvested versus 27.3 million tons from aquaculture). Increasing demand for seaweeds as food products can only be met by further cultivation.

The European Commission recognized that algae (seaweeds and microalgae) were such a promising option for food security that by 2054 their collective cultivation could potentially produce 56 million metric ton of protein, which would then constitute 18% of the global, alternative protein market. The scale of terrestrial agriculture surpasses the production of seaweeds by two orders of magnitude. Land-based agriculture produces about 10 billion tons/year of various products, most of which are plants. Can this figure grow much further, considering the status of arable land, fertilizers and water for irrigation? Can seaweed cultivation grow sufficiently to provide the necessary goods and services for the world's needs? This presentation will outline timelines comparing the principles, practices and potential after millennia of terrestrial vs centuries of marine agronomy.

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ECOPHYSIOLOGIC AND TAXONOMIC REEVALUATION OF THREE CHLOROMONAS SPP. LIVING IN SNOW: COSMOPOLITAN SPECIES TURN OUT TO BE LOCAL ONES

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Melting snow fields in alpine and polar regions are inhabited by snow algae, causing discolouration of snow. In most cases these algae belong to the genera *Chloromonas*, *Chlamydomonas* and *Chlainomonas*. Snow habitats were

colonised several times in the history by these flagellates. In the field, final life-cycle stages, a/planozygotes, are frequently found. Attempts to cultivating them have usually failed since cells are prepared for survival in harsh conditions after complete snow melt. The objective of this study was to explore light requirements, morphology and phylogeny of snow algae thriving in the Tyrolean Alps (Austria), the Giant Mountains (Czech Republic) and the High Tatra Mountains (Slovakia, Poland). The focus was on species of the genus *Chloromonas*, which causes green to orange and reddish snow. Surface cell wall ultrastructure was revealed by scanning light microscopy showing aplanozygotes with flanges or spikes, and thus affiliated with current morphologic descriptions of *Chloromonas nivalis*, *Chloromonas rosae* var. *psychrophila* and *Chloromonas brevispina*. Localities above the timberline are also low light habitats, e.g. in deeper parts of the snowpack, at steep snow slopes in proximity to boulders, and thus are in this respect similar to habitats below the tree canopy. Comparison of the secondary structure prediction of the nuclear marker ITS2 rDNA with available sequences showed that the investigated algae represent several new species based on the compensatory-base change species concept. *In vivo* chlorophyll fluorescence parameters were obtained with a pulse – amplitude modulated fluorometer. Aplanozygotes were physiologically active. Photoinhibition was noticed from 100 to 200 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ upward. Our results suggest that the biodiversity of snow algae in European mountain ranges is still underestimated.

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HYBRIDIZATION OR SPECIATION? UNDERSTANDING CRYPTIC DIVERSITY IN A REPORTEDLY WIDESPREAD SPECIES OF NON-GENICULATE CORALLINE RED ALGAE

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Coralline algae are widespread in all of the world's oceans. Despite their ubiquity, they are a comparatively poorly known group of algae. Ongoing taxonomic changes have meant that the classification and characterisation of these algae have been in constant flux. Recent molecular-assisted alpha taxonomy has demonstrated that coralline algae are

generally not widely distributed across ocean basins and that we have highly underestimated the diversity of these algae, largely due to high levels of cryptic diversity. For South Africa, the reported as widely distributed *Spongites yendoii* has been demonstrated to be no fewer than six different cryptic species. Along the South African west coast, three morpho-anatomically indistinguishable species (here referred to as *Spongites* sp. 6, 7 and 8) have been recognized to occur, all of which have distinct geographic distributions. Based on pbs A sequences, *Spongites* sp. 6 and *Spongites* sp. 8 share seven unique single nucleotide polymorphisms (SNPs). *Spongites* sp. 7 and *Spongites* sp. 8 share six unique SNPs at different base pairs (bp). *Spongites* sp. 8 has five unique bp that it shares with neither *Spongites* sp. 6 nor with *Spongites* sp. 7. *Spongites* sp. 7 has four other bp that are variable. The initial interpretation of these results were that *Spongites* sp. 6 and *Spongites* sp. 7 are different species, but in some places are hybridizing, resulting in *Spongites* sp. 8. Similarly in *rbcL*, *Spongites* sp. 8 is matching either *Spongites* sp. 6 or *Spongites* sp. 7. However, sea surface circulation patterns suggest that we may rather be documenting two separate speciation events, and that separately *Spongites* sp. 8 has given rise to both *Spongites* sp. 6 and *Spongites* sp. 7. We are currently interrogating these suggested opposing events.

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EFFECT OF THE CO₂ INCREASE ON THE GENETIC EXPRESSION OF CARBONIC ANHYDRASE, RUBISCO AND ACTINE IN EMILIANIA HUXLEYI

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This work shows the genetic expression of three genes related to the CO₂ Concentrating Mechanisms (CCMs) (carbonic anhydrase: $\alpha\text{CA 1}$ and $\alpha\text{CA 2}$) and carbon fixation (RuBisCO: *rbcL*), processes responsible for the concentration of dissolved inorganic carbon near RuBisCO and its subsequent transformation to organic carbon in the cell, as well as one structural gene (actine). The response of the marine cocolithophorid *Emiliana huxleyi*, acclimated for at least three weeks, to high CO₂ concentrations predicted for future scenarios of global change (1,000 ppmv de CO₂, High Carbon: HC) and to present CO₂ concentrations

(380 ppmv CO₂, Low Carbon: LC) was tested. Gene expression was measured under HC and LC concentrations using quantitative real time PCR under two different metabolic conditions: in CO₂ acclimated cells with steady metabolic conditions (i.e. after at least 3 weeks CO₂ acclimation) and in cells with perturbed metabolism due to the detention of the aeration during 4 h. The results showed two types of responses when comparing the HC and the LC cells under both metabolic conditions: A down-regulation of the carbonic anhydrase and RuBisCO genetic expression during the CO₂ acclimated steady conditions and an up-regulation in the perturbed cells, associated to the activation of the CCMs after the detention of the aeration. The latter was observed for α CA 1, α CA 2 and actine. Growth responses under similar conditions showed a significant increase under down-regulated conditions and a significant decrease when metabolism was up-regulated. These results indicate the relevance of the metabolic status on the response of marine phytoplankton to ocean acidification.

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RESPONSE OF MAERL (RHODOLITH) ALGAE TO GLOBAL AND LOCAL CHANGES

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Calcareous coralline algae forming maerl beds play a major role as ecosystem engineers in coastal areas throughout the world. They are also subject to strong anthropogenic pressures at a global and local scale, which threaten their survival. The aim of this work was to give insights on the future of maerl beds in a context of global and local changes. The effects of rising temperature (ambient, +1.3°C [RCP4.5] and +2.6°C [RCP8.5]), decreasing pH (ambient, -0.15 pH units [RCP4.5] and -0.33 pH units [RCP8.5]) and nutrient concentrations (N and P) on maerl species (*Lithothamnion corallioides*, *Phymatolithon calcareum*, *Lithophyllum incrustans*) was evaluated in the laboratory under winter and summer conditions. Measurements of metabolic rates (primary production, respiration and calcification) were performed on the three maerl species.

Nutrient enrichment had a positive effect on photosynthesis at low pH and high temperature under winter conditions. In summer, no significant differences were observed among treatments in terms of photosynthesis. At low pH and high temperature, calcification was favored by nutrient enrichment for both seasons.

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THE CULTURE COLLECTION OF ALGAE AND PROTOZOA (CCAP): CURRENT EFFORTS AND VISION TO UNDERPIN THE RISE OF ALGAL AQUACULTURE

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In the last few years, algal cultivation has experienced a phenomenal level of development in Europe, and with it, the need for protocols to ensure the viability and functionality of the strains selected for farming has increased. In response the CCAP, as a Biological Resource Centre, is working on several strands of research to underpin the sustainability of the sector.

In recent years, methodological development has increased the diversity of microalgal taxa that can be cryogenically stored successfully. Research has explored the functionality and stability of preserved materials, along with the implications of conserving algal-based consortia. In addition, collaborative capacity-building projects have resulted in new cryopreserved algal collections in Sweden, Belgium and Brazil.

To support the development of disease management and biosecurity in micro- and macro-algal production facilities, protocols to isolate, maintain and cryopreserve pathogens of commercially important algae (e.g. *Haematococcus*, diatoms, red and brown seaweeds) have been developed. This effort is enabling considerable improvement in knowledge of the taxonomy, life cycle and physiology of these poorly-known pathogens and accordingly, supports the design of control methods to eradicate diseases in algal farms.

As part of the GENIALG project, the CCAP is planning to establish an ambitious seaweed “seedbank” to ensure that wild genetic diversity is documented, conserved, and made publicly available for biotechnological and research purposes. A pan-European seedbank of the kelp *Saccharina latissima* will ensure not only the maintenance of a diverse number

of strains, but also the preservation of their functionality, and where technically possible, genetic stability. Clonal gametophytes collected from defined geographic zones will be progressively accessed in the Collection, and efforts will be made to develop cryopreservation, guarantee strain stability and increase the robustness of maintenance protocols. Phenotyping and genotyping data will be gathered and made available to users worldwide via the CCAP Knowledgebase.

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**A NOVEL SPECIES OF PARASITIC
LOBULOMYCETALES INFECTING
ASTERIONELLA FORMOSA:
CULTIVATION, INFECTIOUS CYCLE
AND CRYOPRESERVATION**

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Parasitic Chytridiomycota (chytrids) are ecologically significant in various aquatic ecosystems, notably through their roles in controlling bloom-forming phytoplankton populations. They are a proven link in the food web, facilitating the energy transfer from inedible algae to higher trophic levels. The diversity and study of these obligate parasites, whilst critical to understand the interactions between pathogens and their hosts in the environment, has been hindered by challenges inherent to their isolation and stable long-term maintenance in laboratory conditions. In the present study, we isolated an obligate chytrid pathogen on its host, the freshwater bloom-forming diatom *Asterionella formosa* and characterised its infectious cycle in controlled conditions. Phylogenetic analyses based on 18S, ITS1-5.8S-ITS2 and 28S rDNA revealed that the chytrid is indeed a new species belonging to a novel clade within the Chytridiomycota. We describe the new species, investigate its host range and present a robust and simple assay that assesses both the infection success and the viability of the host. We also validate a cryopreservation method

for stable and cost-effective long term storage, and reach full recovery of the chytrid infectious characteristics post-cryopreservation. A quantitative assessment of the chytrid infectivity post-cryopreservation demonstrates its full recovery after thawing, thus guaranteeing the long term availability of material for future study of this interesting novel organism. All of the above tools establish a new gold standard for the isolation and long-term preservation of pathogenic aquatic chytrids, thus opening new perspectives to investigate the undiscovered diversity of these organisms and their physiology in a controlled laboratory environment.

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**FATTY ACID METHYL ESTER, PHTHALIC
ACID ESTERS AND SCREENING BIOACTIVE
PHYTOCHEMICALS OF GRACILARIA
SALICORNIA, PADINA BOERGESENII
AND POLYCLADIA MYRICA FROM PERSIAN
GULF**

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Pathogenic and multidrug-resistant bacteria are causing increased concern in healthcare institutions worldwide. In the present study MeOH and 12aqueous extracts of *Gracilaria salicornia*, *Padina boerGESENII* and *Polycladia myrica* were assessed against the Gram-positive bacteria *S12taphylococcus aureus* (MRSA), *Micrococcus luteus* and the Gram-negative 12bacteria *Pseudomonas aeruginosa* and *Escherichia coli*. The extracts were analyzed by GC-MS using column chromatography and reversed- phase high-performance liquid chromatography. The major chemical constituents are nonanoic acid, bicyclo[3.1.1]hept-2-ene-2-methanol, 6,6-dimethyl- (Myrtenol (2-Pinen-10-ol)), cyclohexadecane, 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester, cyclic hydrocarbon, hexadecanoic acid, methyl ester and octanoic acid. The extracts were characterized by substantial levels of terpenes, diterpenes, alcohols, hydrocarbons, cyclic hydrocarbons, esters and fatty acids. Screening of the extracts showed low to high antibacterial activity against

tested bacterial strains (34.3 ± 0.6 – 5.3 ± 0.58 mm). These data indicate that they have potential for application in the topical and systemic treatment of drug-resistant bacterial infections.

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HALOPHILISM IN SOME INDIGENOUS STRAINS OF NOSTOC FROM ARIDISOLS OF HISAR, INDIA

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Performance of four indigenous strains of Nostoc isolated from aridisols of Hisar was studied under controlled conditions with a view to exploit these strains as biofertilizers under varying levels of soil salinity, alkalinity and water stress for bioremediation of usar soils. The cyanobacterial cultures were exposed to salinity stress (55, 110 mM), corresponding osmotic stress (180, 360 KPa) and different pH levels (7.5, 8.5, 9.5). The magnitude of halotolerance varied among the cyanobacterial species and halophilism was favoured by alkaline pH. Although these species showed osmo-tolerance as revealed by high biomass, sugar accumulation and exo-polysaccharide (EP) production were associated only with NaCl stress. Halophilic behaviour, measured in terms of biomass and chlorophyll in the presence of NaCl, was found to be associated with accumulation of soluble sugars and ion uptake pattern. Ion uptake (Na^+ , K^+) was directly related to EP production by the algae. A major proportion of cell bound Na^+ was found to be adsorbed extracellularly to the exo-polysaccharides thereby maintaining lower intracellular Na^+ concentrations in the strains showing higher halophilism.

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CHEMICAL COMPOSITION AND BIOACTIVITY POTENTIAL OF ULVA INTESTINALIS: ANTIBACTERIAL AND ANTIOXIDANT

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Antibacterial activities of methanol and aqueous and aqueous-methanol extracts of *Ulva intestinalis* were assessed against Gram-positive bacteria *Staphylococcus aureus* and *Micrococcus luteus* and also Gram-negative bacteria *Pseudomonas aeruginosa* and *Escherichia coli*. Extracts showed low to high antibacterial activity against tested bacterial strains. However, the antibacterial activities of extracts were not comparable to the positive controls. Antioxidant activities of three extracts of algae were evaluated using DPPH (2,2-Diphenyl-1-picrylhydrazyl) at the concentration of 1,875–6,000 mg/L. In this method, MeOH extract possessed the highest antioxidant activity at the concentration of 15,000–60,000 mg/L, whereas aqueous extract possessed higher antioxidant activity at the concentration of 1,875–7,500 mg/L in comparison to MeOH extract. In addition, the antioxidant activity of aqueous extract was the lowest. The major Chemical constituents of MeOH and aqueous-methanol extracts included dimethyl sulfide, tetradecamethyl-cycloheptasiloxane, 2-propenoic acid, 2-dodecanone.

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INTERACTIONS BETWEEN ENERGETICS AND COMPOSITION

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The chemical composition of algae determines the energy cost of synthesis and maintenance of the biomass. Changes in environmental factors such as temperature, photosynthetically active radiation and ultraviolet radiation

influence the composition of organisms when the supply of essential elements in a form that the alga can use is not limiting growth. A restricted supply of one or more essential elements decreases the growth rate and also alters the composition of the organism with implications for the energy cost of biomass synthesis and maintenance. For phytoplankton in deep water bodies, e.g. the open ocean, a decrease in the mixing depth of the upper mixed layer with increasing temperature of the surface ocean increases the mean incident photosynthetically active radiation and ultraviolet radiation. These changes in irradiance interact with decreased nutrient supply from the deep ocean as a result of the larger temperature difference across the thermocline.

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APPLYING HIGH THROUGHPUT SEQUENCING (HTS) ON SNOW ALGAE: CURRENT STATE, KNOWLEDGE GAPS AND FUTURE PERSPECTIVES

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Cryoflora consists of specialized microalgae which are mainly Chlorophyta, with an emphasis on Chlamydomonadaceae. Blooms cause snow discolorations in mountainous or polar habitats during spring and summer. Exploration of the biodiversity of cryoflora has been hampered by weak morphologic details of spore stages dominating the snow surface and the fact that many taxa were not yet culturable for life cycle studies. In the last few years, HTS has been successfully performed on environmental samples, including characterisation of biocoenosis data of bacteria and fungi living in snow. It allows an in-depth characterization of communities and reveals also low abundance taxa. However, this complement to classic sequencing raised several questions, especially taking the poor resolution of the 18S rDNA marker gene in the Chlamydomonadaceae into account. In addition, the short read length of HTS can only target a fraction of the marker genes, which must be chosen with care.

In our approach, we analysed four snow algae from the Austrian Alps (*Chlamydomonas nivalis*, *Chloromonas brevispina*, *Chlainomonas* sp. and *Chloromonas rosae* var. *psychrophila*) by HTS and Sanger sequencing, using 18S and ITS2 genes. We found that evaluation of the data was highly affected by the quality of the databases, which depend on the submission of references sequences derived from classic sequencing of single taxa. Furthermore, the multitude of sequences derived from HTS can reveal the existence of oligotypes within one taxon. In sum, we attempted to show the current state and limits for designating OTUs and thus estimating the true biodiversity of Alpine cryoflora.

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ENHANCEMENT OF PHENOLIC ACID AND FLAVONOID IN PLANTAGO MAJOR BY EXTRACT OF CYANOBACTERIA

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Numerous reports are available in the literature regarding cyanobacteria and nitrogen fixation and soil fertility in paddy fields. There are also many reports on the effects of cyanobacteria on growth parameters of different crop plants, but reports on medicinal plants are scarce. In this study 43 cyanobacterial taxa were isolated and identified from the soil in which *Plantago major* L was growing. The identified taxa belonged to the order Nostocales and were placed in the families of Nostocaceae and Rivulariaceae. A seed germination test was carried out for each algal suspension in order to select the most effective species. Based on the seedling growth test, three domestically purified species of heterocystous cyanobacteria were chosen for this study: *Anabaena vaginicola* F.E. Fritsch & Rich, *Cylindrospermum michailovskoense* Elenkin and *Nostoc spongiaeforme* var. *tenue* C.B. Rao have a significant effect on growth parameters of treated plants of *Plantago major* L. Growth of plants was evaluated by measuring growth parameters such as leaf number, root length, the number and length of

inflorescence, and leaf surface as compared to control. In addition, phenolic acids were analyzed by HPLC, and paracomaric acid was identified as the dominant compound in ethanol/water extract (50%). The analysis of data showed that the highest amount of paracomaric acid was estimated in the plant inoculated with *Nostoc spongiaeforme* var. *tenuis* ISB63. However highest total phenol and flavonoid was found in *Platago major* L that was inoculated with *Cylindrospermum michailovskoene*.

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METAGENOMICS AND METATRANSCRIPTOMES OF OCEANIC COMMUNITIES

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The *Tara* Oceans expedition circumnavigated the globe over a multi-year period, collecting samples in the sunlit ocean from over 150 individual stations. For eukaryotic plankton ranging from 0.8 μm –2 mm, we produced three types of sequencing data for each sample, from the following sources: (1) metagenomes: whole-sample genomic DNA extraction; (2) metabarcodes: PCR amplification of the V9 hypervariable region of the 18S ribosomal locus; (3) metatranscriptomes: polyadenylated mRNA selection of whole-sample RNA extraction. We present analyses that compare and contrast each data set. First, based on sequence similarity among metagenomes, we partition the oceans into regions inhabited by similar plankton communities. For smaller organisms, these regions are consistent with the provinces of Longhurst based on biogeochemical data, but this relationship breaks down above 20 μm , likely reflecting the contrast between shorter-lived, smaller primary producers and longer-lived, larger heterotrophs in the ocean. Next, we analyze taxonomically-identified metabarcode data, as a complement to largely anonymous metagenomes, to study the patterns of change within communities across an example region, the North Atlantic. For plankton sized 180 μm –2 mm, we demonstrate a transition from Collodaria-characterized communities to Metazoa-characterized communities along the Gulf Stream. Finally, to measure the activity of plankton communities, we apply a phylogenetic read placement approach to map metatranscriptomic sequences onto a set of 250 conserved eukaryotic gene trees. We find that, globally, metabarcodes and metatranscriptomes show a similar representation of major eukaryotic lineages. We close with an example of a specific gene, SIT, which transports extracellular silicon into organisms that construct silica-based structures, such

as the frustules of diatoms. Diatoms are responsible for the majority of global SIT transcription, especially in the colder waters of the Southern Ocean, but their dominance is supplanted by choanoflagellates and animals in the Mediterranean and the Indian Ocean.

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EVOLUTIONARY HISTORY AND DIVERSITY OF MEDITERRANEAN CORALLINE ALGAE: HOW MUCH DO WE KNOW?

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In the Mediterranean Sea coralline red algae play a particularly important role, as their calcified thalli provide hard substratum in several benthic biocenoses covering large portions of rocky bottom (i.e. coralligenous concretions, rims of *Lithophyllum byssoides*, rims of articulated corallines). Since its opening 250 Myr ago, the Mediterranean has undergone a complex oceanographic history, due to geological and climatic events such as the Atlantic opening, the isolation from the Indian Ocean, and the Messinian Salinity Crisis, which determined phases of connection and separation from the adjacent oceans. These events profoundly affected the evolutionary history of Mediterranean marine organisms, fueling a high biological diversity. Paleontological evidence indicates that corallines have been major bioconstructors in the Mediterranean for a long time and that some common species have occurred in the basin for at least 10-11 Myr (*Lithophyllum dentatum*, *L. incrustans*, *L. pustulatum*). The limited amount of molecular data available has hindered our understanding of the evolution and diversity of this group, but recent studies provide major insights in these topics. The recent discovery of *Pneophyllum cetinaensis* shows that the Mediterranean is the only geographical region in which coralline algae have successfully crossed the border between sea and freshwater, a transition that probably took place during the last glaciation. *Lithophyllum byssoides*, one of the few genuinely intertidal Mediterranean seaweeds, has a high haplotypic diversity and Mediterranean populations probably represent

a different species from Atlantic populations, a pattern observed for many animal species. Similarly sharp haplotypic differentiation between Mediterranean and Atlantic populations has been documented for species of *Mesophyllum* and *Corallina*. *Lithophyllum cabiochiae*, the main bio-constructor of coralligenous concretions, represents a highly diverse complex of species, rather than a single species. These studies suggest that Mediterranean corallines represent a more evolutionarily complex group than believed so far, worthy of further detailed investigation.

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DIFFERENTIAL GENE EXPRESSION OF ZYGNEMA CIRCUMCARINATUM IN RESPONSE TO DESICCATION STRESS

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Zygnema is a conjugating green alga (Zygnematophyceae) which mostly colonizes hydro-terrestrial habitats. The alga is able to cope with various abiotic stress factors which are prevalent in terrestrial ecosystems, such as high UV-radiation, low nutrient availability and desiccation. We exposed differently matured cultures of *Z. circumcarinatum* (SAG2419), which was isolated from the sandy shore of the river Saalach near Salzburg, to continuous desiccation until the effective quantum yield of photosystem II dropped to zero. To study the influence of pre-akinetes formation on water stress tolerance, we compared a desiccated liquid culture (one month old vegetative cells) to desiccated pre-akinetes (seven months old) grown on solid medium. Differential gene expression analysis revealed a stronger response to dehydration treatment in the liquid culture, with 2,494 transcripts being regulated, compared to the 849 showing transcriptional changes in the pre-akinetes. Several transcripts encoding photosystem I and II are repressed in order to reduce ROS (reactive oxygen species) production. Furthermore, desiccation is counteracted by an increased formation of sucrose in the cells which acts as a low-molecular-weight osmolyte and protects membranes and proteins. In addition, parts of the glycerol- and glycerophospholipid metabolism exhibit enhanced expression, suggesting membrane modifications. Aquaporins transcription is high to increase the membrane permeability to water as a precaution against rehydration induced damage. Finally, ROS scavenging, protection and repair mechanisms are

activated. For example, we found a number of late embryogenesis abundant (LEA) proteins to be up-regulated during desiccation treatment. Our findings indicate that culture age and conditions greatly influence the acclimation to water stress. In natural habitats, this plasticity enables *Zygnema* to cope with seasonal fluctuations.

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POLAR BIOLOGICAL SOIL CRUST ECOLOGY – IDENTIFICATION OF GENERA USING CLASSICAL AND MOLECULAR APPROACHES

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Biological soil crust (BSC) communities are important primary producers in polar ecosystems such as the Arctic and Antarctica. Moreover, these biocenoses fix nitrogen, prevent erosion and promote biodiversity in these sparse habitats. BSCs are comprised of numerous different organisms such as prokaryotic and eukaryotic algae, protozoa, fungi, lichens, bacteria and bryophytes. We studied algal genera in different soil crusts collected at the Arctic Spitsbergen and the Antarctic Livingston Island. The algae were identified by means of cultivation-based methods and molecular surveys on metatranscriptomic datasets which were subsequently compared. We limited our study to Klebsormidiophyceae, Chlorophyceae, Trebouxiophyceae, Xanthophyceae and Cyanobacteria, which are some of the most abundant algal groups in BSC. Our results indicate a larger diversity in the molecular datasets compared to the number of successfully cultivated genera. This underestimation of biodiversity occurs, most certainly, due to the limited culturability of many organisms. However, molecular approaches also possess several drawbacks such as lack of reference sequences. Furthermore, comparisons within, as well as between, methodologies stand and fall with homogeneity of the samples, which is difficult to achieve for environmental samples. Overall, we recommend a combined

approach in order to study the biodiversity of BSCs more thoroughly.

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ISOLATION OF FLAVONOID FROM SARGASSUM CRISTAEFOLIUM AND THE EFFECT ON MULTICELLULAR ANTIPROLIFERATION ACTIVITY

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Sargassum cristaefolium has been isolated for flavonoids, and screened for antiproliferation activity using the sea urchin embryo. High rise maceration method was used to gradually extract the seaweed with chloroform, acetone, and methanol. The three extracts obtained were tested using BSLT (Brine Shrimp Lethality Test) with *Artemia salina* and showed a different value of LC50 for each solvent. Methanol extract of *Sargassum* has several phytochemical compounds, among others, flavonoid, flavon, alkaloid, terpenoid and steroid, but flavonoid was the most important. A further experiment was performed using TLC (Thin Layer Chromatography) using a different ratio of toluene, ether and acetic acid and then by a HPLC qualitatively. Analyses by UV-Visual with a shear reagent and infrared spectroscopy (IR) suggested that the flavonoid compound contained in the methanol extract was 5,6,7 dihydroflavanol with benzene rings. The methanolic fraction was tested for its antiproliferation activity using sea urchin embryo of *Temnopleurus alexandri* and showed multicellular growth inhibition.

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WHAT IS THE IMPACT OF NITRATE LIMITATION ON THE CULTURE MICROBIOME OF OLEAGINOUS NANNOCHLOROPSIS OCULATA?

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Switching microalgae cultures from nitrate replete (to promote growth) to delete conditions (to instigate neutral lipid synthesis) is common practice in microalgae biodiesel production. However, this step is done without due consideration of the potential impacts on the culture microbiome. Homeostasis between the cultured microalgae and their associated bacterial communities tends to promote stable culture conditions. When homeostasis is disrupted, for instance by sudden shifts in the nutrient environment, the potential exists for opportunistic and/or pathogenic bacteria to establish, which may inadvertently destabilise culture operations. Remarkably, given the enormous investment in microalgae biodiesel production, this part of the culture cycle has received only cursory attention. Using next generation sequencing, we directly address this by examining the response of bacterial communities associated with the oil-rich microalga *Nannochloropsis oculata*. Interactions between microalgae growth, lipid yield and bacterial diversity were investigated under contrasting nitrate conditions. It is hoped that by understanding the microbial ecology of the culture, more subtle 'bottom-up' approaches to biofuels culture management and manipulation may be designed that may increase operational robustness and reduce culture crashes.

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THE TOUGH AND TENACIOUS: THRIVING IN NATURAL ANALOGUES OF WARMER AND ACIDIC TROPICAL MARINE WATERS

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Sea surface temperatures, already warmest in the Indo-Pacific, continue to rise while pCO₂ surpasses glacial-interglacial concentration fluctuations in the past 2 million years. The resilience of the Coral Triangle ecosystems is threatened as CO₂ from the atmosphere diffuses, dissolves and acidifies the ocean compounding the problem of warming temperatures. Natural analogues of warm and acidic seawater exist in Mabini, Batangas, Philippines, where faults and calc-alkaline volcanoes border the area in the Verde Island Passage Marine Corridor, the heart of the center of marine biodiversity. Coastal waters in Mabini, particularly in a site called Mainit feature bubbles emanating from underwater bedding planes, faults, joints, and sediments. Interestingly, the site is characterized by warm temperatures (30–60°C), high seawater CO₂ (>6,000 ppm), and acidic waters (as low as pH 5.7). Despite the harsh conditions, marine plants (seagrass and seaweeds), corals, fish, and other organisms continue to thrive within the vicinity in this challenging environment. We describe the conditions and preliminary survey of marine life done in the area.

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RED, BLUE, AND GREEN, AND SHADES IN BETWEEN: DIVERSITY AND PLASTICITY IN COLOUR EXPRESSION IN THE LIFE CYCLE OF KAPPAPHYCUS

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The Philippines is considered the center of *Kappaphycus* biodiversity in the world. Currently, six *Kappaphycus* species have been recorded in the Philippine archipelago. Different strains of cultivated eucheumatoids exhibit different phenotypes, with colouration being the most distinct difference. In this study, fertile wild *Kappaphycus alvarezii* specimens were collected on the reef flat of Borata, Guiuan, Eastern Samar, Philippines. The parental tetrasporophyte was a bicolor i.e. brown and green, while the female gametophyte with cystocarps was dark brown. Tetraspores

and carpospores from respective parental individuals were separately release and cultivated until the next life history phase, i.e. the next generation of gametophytes and tetrasporophytes, respectively. After 2 years cultivation in the laboratory and subsequently in an outdoor land-based nursery, the resulting gametophytes from a bicolor parental tetrasporophytes expressed a host of colours from red, brown, and green, and shades in between. On the other hand, the resulting tetrasporophytes from a dark brown female gametophyte (with corresponding dark brown cystocarp) were dark green. Phytochrome-regulated sensory system in photosynthetic organisms controls the production of proteins used to capture light for photosynthesis. The control of pigmentation by light is called complementary chromatic adaptation (CCA) which is photoreversible. However, considering that the wild specimens were collected on the same reef flat and the culture and environmental conditions of the progenies in the laboratory and in the outdoor nursery are comparable, the expression of particular colouration may be adaptive and within the species' natural pool of genetic variability.

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MORPHOLOGICAL PLASTICITY AND ACCLIMATION LIMITS OF FUCUS EVANESCENS ALONG THE SALINITY GRADIENT OF THE BALTIC SEA

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In the Baltic Sea proper, the genus *Fucus* is represented by three species: *Fucus vesiculosus*, *F. serratus* and *F. radicans*, the latter being seen as an example of ongoing speciation in this rather young ecosystem. In the transition area to the North Sea two more species, *F. spiralis* and *F. evanescens* are found. Whereas *F. spiralis* is common in the North Sea and only remarkable because of its rather low penetration into the low-saline parts of the Baltic Sea, *F. evanescens* is regarded as an invader, which has established in the Baltic Sea during the last decades. Since the first discovery of *Fucus evanescens* in the Öresund area during the 1950s, this species has extended eastwards into the Baltic Sea until Stralsund, indicating a gradual adaptation to the low-saline conditions behind the Darss Sill. However, little is known yet about the acclimation limits and the distribution range

of this species. First described from a site in the Pacific Ocean, which is at the edge of its distribution range, the acclimation potential of *F. evanescens* is poorly understood. Moreover, morphological variability makes it hard to recognize this species in the field, resulting in different opinions about useful characteristics for distinction from related furoid species such as, e.g., *F. distichus*. The work presented here investigates the acclimation limits of different *F. evanescens* populations with respect to habitat salinity and describes their morphological plasticity in order to estimate the invasion potential as well as to identify reliable characteristics for species recognition.

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BIOFABRICATION OF GOLD AND SILVER NANOPARTICLES USING FRACTIONED CELLULAR COMPONENTS OF EUKARYOTIC ALGAE AND CYANOBACTERIA

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In the present communication fractioned cellular components such thylakoids/chloroplasts, carotenoids, proteins and polysaccharides of pro- and eukaryotic algal cells were used individually against auric chloride and silver nitrate solutions to search for an efficient reducing agent to synthesize monodisperse gold (GNP) and silver (SNP) nanoparticles. These fractioned cellular components were extracted from pure cultures of *Anabaena sphaerica*, *Leptolyngbya tenuis*, *Chlorococcum infusionum* and *Rhizoclonium riparium*. The protein and carotenoids extracts of all experimental taxa synthesized both GNP and SNP whereas, extracted polysaccharides from green algae and cyanobacteria responded differently. Polysaccharides from cyanobacteria showed a positive response in nanoparticle synthesis, whilst green algal polysaccharides remained ineffective in the reaction mixture. The particles synthesized by extracted polysaccharides, protein and carotenoids showed marked poly-dispersity having different shapes such as spherical, oval, triangular and hexagonal. Isolated chloroplasts/thylakoids from the selected strains were found to be efficient metal ion reducing agent and produced only spherical shaped GNP and SNP at pH 4. Synthesis of monodisperse GNPs by isolated thylakoids from *Anabaena* and chloroplasts from *Chlorococcum* was also recorded at pH 9. In UV-vis spectroscopy, GNP showed maximum absorbance at ~530–540 nm and sharp peak appeared at ~420 nm for

SNP. Transmission Electron Microscopy determined the size and shapes of the particles. In X-ray diffraction analysis four intense peaks appeared at 38.2°, 44.5°, 64.8° and 77.8° which confirmed the crystallographic nature of the synthesized particles. Any morphological change in metal treated biomass was investigated in detail using Scanning Electron Microscopy and the fluorescence properties of particle loaded cells was studied using Fluorescence Microscopy. It emerged from this study that biosynthesis of monodisperse GNP is possible by varying pH.

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THE N SOURCE INFLUENCES C ACQUISITION, FIXATION AND ALLOCATION IN THE CYANOBACTERIUM SYNECHOCOCCUS SP. ONLY WHEN ENERGY IS LIMITING

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The energy required for the assimilation of nitrate is substantially different from that needed for the assimilation of equal amounts of ammonium. We hypothesize that changes in the availability of the two N forms may affect the partitioning of energy to C acquisition, assimilation and allocation, ultimately determining differences in the algae rate of growth and cell composition. This would become obvious only when energy is limiting.

In order to test this hypothesis, we used the cosmopolitan cyanobacterium *Synechococcus* sp. UTEX 2380, under conditions of either N or light limitation, in the presence of either nitrate or ammonium.

Our results show that, when energy was the limiting factor, *Synechococcus* sp. grew faster in the presence of ammonium than in nitrate, and had higher C, N, and S cell quotas, biomass productivity and photosynthetic rates. Also the photosynthetic affinity for dissolved inorganic C and the size of the internal C pool were higher in energy-limited ammonium grown cells. These differences were mostly not present when energy was sufficient and N was the limiting factor. We interpret these results as an indication that, under energy limitation, the use of the least expensive N source allows a greater investment into energy-dependent inorganic C acquisition and the increased availability of

cell C influences its allocation and growth. The ecological consequences of these findings will be discussed.

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INFECTION AND TREATMENT- RESISTANT OF THE ICE-ICE DISEASE IN AGAROPHYTE, GRACILARIA FISHERI (GRACILARIALES, RHODOPHYTA)

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The agarophyte, *Gracilaria fisheri* cultivated in a pond was attacked by Ice-Ice disease. This study was aimed at developing a technique for recovering production in the pond. Microorganisms were isolated and inoculated in laboratory. The infected tissues were treated by dipping into high concentration and soaking in low concentration of five chemical substances: chlorine, potassium permanganate, iodine, oxytetracycline hydrochloride, and gentian violet. The result was that five species of bacteria were found in those tests: *Vibrio* sp.1, *Vibrio* sp.2, *Micrococcus* sp., *Planococcus* sp. and *Pseudomonas* sp. Only the gram- positive coccus bacteria, *Micrococcus* sp. and *Planococcus* sp. caused the regular thalli of the seaweed to be pale and broken as had been found in the cultivation ponds. These bacteria caused this irregularity only in the experiments at 30°C not at 20 and 25°C. The infected fragments of the seaweed were cultured at different temperature and salinity levels, 20, 25 and 30°C, under 70 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ at 20 ppt of salinity with 12:12 hours of light: dark period. In tissue at 20°C the infection stopped and the scars recovered, this did not occur in tissue at 25 and 30°C. The infected tissue from 500 ppm iodine, 1,000 ppm oxytetracycline hydrochloride and 20 ppm gentian violet at 20°C showed the highest regrowth of tissue cultured in iodine 93%. The ice-ice symptom could be observed in he tissues cultured at 25 and 30 °C. Therefore, seedlings from infected thalli of *Gracilaria fisheri* could be used for propagation, cultured in low concentration of 20 ppm iodine.

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PHYTOPLANKTON COMMUNITY CHARACTERISTICS OF DADONG SEA IN AUTUMN 2016

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To understand the the status of community structure and diversity of phytoplankton in waters in Dadong Sea of Hainan Province, investigations on phytoplankton in eight stations were carried out in September 2016. Species diversity was evaluated using Shannon-Wiener diversity index (H'), Pielous species evenness (J') and Berger-Parker dominant degree (Y). In total 74 species (including variety) in 32 genera were identified, of which 48 species in 22 genera belonged to Bacillariophyta, 25 species in 9 genera for Pyrrophyta and 1 species in 1 genus for Cyanophyta. Most of the phytoplankton were diatoms. The dominant species were *Chaetoceros coarctatus*, *Ceratium furca* var. *furca*, *Achnanthes brevipes* var. *angustata*, *Coscinodiscus wailesii*, *Leptocylindrus danicus*, *Rhizosolenia styliformis* var. *latissima*, *Trichodesmium erythraeum*, *Ceratium fusus* var. *fuscus*, *Hemiaulus membranaceus*. The cell abundance of phytoplankton ranged from 0.65×10^4 to 41.93×10^4 cells-L⁻¹, with an average of 10.80×10^4 cells-L⁻¹. H' value was very high, ranging from 2.28 to 3.95. J' value ranged from 0.60 to 0.81, Species diversity was very rich. According to the composition and structure of phytoplankton communities, the water quality in this area was good.

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THE INTERACTION BETWEEN A GREAT POND SNAIL (LYMNAEA STAGNALIS) AND BLOOM-FORMING MACROALGAE ULVA FLEXUOSA

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Cosmopolitan species of the genus *Ulva* (Ulvaceae, Chlorophyta) which populate the littoral zone of marine habitats constitute a staple diet for a variety of organisms, particularly snails, shellfish, polychaetes, and birds. Occurrence of *Ulva* species (e.g., *U. flexuosa* and *U. prolifera*) has also been observed in freshwater inland ecosystems that have no contact with saline water. However, the influence of the development of macroalgal mats of *Ulva* on indigenous organisms in limnic ecosystems has not been established. This study investigates the trophic relationships between *Ulva flexuosa* and one species of snail from freshwater habitats in central Europe. During summer, the great pond snail (*Lymnaea stagnalis*) consumed *Ulva* as a source of nutrition even when other algae and plants were available. *L. stagnalis* consumed an average of 100 mg of *Ulva* thalli per day. This exceeded the consumption of an alternative food source, the shoots of *Elodea canadensis*. *Ulva* thalli are more actively consumed by great pond snails than *Elodea* shoots, and this is expressed in terms of the differences of biomass consumption. It was also observed that the interior of the monostromatic tubular thalli of *U. flexuosa* serves as a protective shelter for juvenile great pond snails.

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

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The diversity of eukaryotic algae along a soil chronosequence in a glacier forefield of Fildes Peninsula (King George Island Maritime Antarctica) and from a German

agricultural field has been studied using two complementary culture-independent approaches, cloning and Illumina MiSeq (2 × 250) paired ends sequencing targeting the ITS2 rRNA gene. Group-targeted PCR amplification was required to minimize biases in biodiversity assessments because eukaryotic algae often seemed to be underrepresented in the DNA extracts from the soil samples. PCR primer pairs targeting various green algal classes (e.g. Trebouxiophyceae, Klebsormidiophyceae) and the stramenopile Xanthophyceae were employed. With those for the Xanthophyceae ITS2 rRNA genes of no other organisms were recovered. Long ITS1-5.8S-ITS2 amplicons were separated by cloning and sequenced. For Illumina MiSeq (2 × 250) paired-ends sequencing suitable shorter fragments comprising full ITS2 region were re-amplified using unspecific primers. We developed a pipeline for OTUs extraction and taxonomical assessment that combines different established software tools and own scripts. NGS approach revealed a high algal diversity of 741 green algal and 162 xanthophyte OTUs (97% identity level, OTUs with >0,005% sequences in sample). Across the chronosequence total numbers of OTUs varied from 343 to 525, while only 176 were recovered from the field. Cloning/sequencing resulted in a total of 169 OTUs from Antarctica and 49 from the field, but no saturation was reached. The scarcity of reference sequences available for the ITS2 region prevented assignment of 1/3 OTUs to a precise taxonomic position. Despite the NGS approach revealing a high diversity of xanthophytes, longer ITS2 sequences (>380bp) as recovered by clone libraries for this algal group were absent, indicating that the xanthophycean diversity as revealed by NGS may have been biased. Ubiquitous species already known from geographic regions other than Antarctica as well as species probably confined to Antarctica, i.e. so far recovered only by our study, were revealed.

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RECIPROCAL INTERACTIONS SHAPE THE STRUCTURE AND FUNCTIONING OF DIATOM-BACTERIA CONSORTIA IN MARINE BIOFILMS

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The long history of coexistence of diatoms and bacteria in aquatic environments has led to the evolution of complex antagonistic and synergistic interactions between both groups of organisms. In order to understand the nature of these interactions and how they affect the functioning of diatom-bacteria consortia we first need to understand what processes shape diatom microbiomes, and how this in turn affects diatom fitness. In a series of experiments with diatoms and bacteria from marine intertidal biofilms, we investigated how host identity, environment and bacterial source community affect diatom microbiome assembly. We further tested how diatom microbiomes affect the growth and competitiveness of their hosts. Our experiments revealed that host identity (both at the specific and intraspecific level), environment (particularly nutrient availability) and bacterial source community all significantly impacted the taxonomic composition of microbiomes recruited by (axenic) diatoms. Interestingly, host identity (and even presence of diatoms) had no effect on the functional diversity of the microbiomes (as assessed with Biolog Ecoplates), which mainly depended on nutrient availability. Addition of bacterial communities to axenic diatoms initially strongly reduced diatom growth in all experiments, but the extent of growth reduction differed between diatom species. This species-specific effect on diatom fitness had important implications for the outcome of competitive interactions between diatom species. The growth of the diatoms recovered gradually after several rounds of reinoculation of the co-cultures, suggesting a shift from antagonistic (e.g. competition) towards more neutral or mutualistic interactions.

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BOOM AND BUST PATH OF GRACILARIA: LOSS OF BACTERIAL “FRIENDS” CAN LIMIT THE INVASION SUCCESS OF A SEAWEED HOST?

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Invasive species are one of the principal components of global change along with ocean warming at the global scale, or overfishing and deoxygenation at the regional scale. Seaweeds represent up to 40% of all introduced marine species and some seaweeds can significantly affect

the composition and functioning of marine benthic communities. Within ten years of its first discovery in the Kiel Fjord in 2005 the East Asian red seaweed *Gracilaria vermiculophylla* has spread approximately 100 km eastward and 120 km northward along the German Baltic Sea coast, now inhabiting many lagoons and sheltered bays between the German-Danish border and Neustadt. During the first two years after its discovery *Gracilaria vermiculophylla* increased its biomass in the Kiel Fjord massively. However, this was followed by a sudden decline in late summer 2008, when the alga decayed in nearly all inhabited parts of the bay within few weeks. Co-cultivation of healthy *Gracilaria* from unaffected environments with small amounts of decaying material from the Kiel Fjord in laboratory assays demonstrated that the decay was apparently caused by an infectious disease. Thus, 59 different species of epibacteria isolated from *Gracilaria* were tested for their capacity to induce decay in a bleaching assay. Out of these, three were found to induce the disease, while 19 others significantly reduced the risk of decay and were thus protective. When protectors and pathogens were tested together, the protective strains fully prevented the negative impact of the bleachers, hinting at the presence of an associational defence offered by *Gracilaria's* epibacteria. Presence of such an associational resistance was also supported in a follow-up bioassay where surface extract of *Gracilaria* and its associated microbiome attracted the beneficial strains, but deterred the detrimental ones. Thus, we suggest that the breakdown in 2008 was due to a collapse of such associational resistance provided by bacterial partners.

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WHOLE GENOME SEQUENCING OF TWO CLOSELY RELATED BUT MORPHOLOGICALLY DISTINCT CLONES OF CENTRIC DIATOM HYDROSERA

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The centric genus *Hydrosera* has a rectangular outline in girdle view, and multipolar (e.g. bi-polar, triangular, pentagonal to hexagonal form) valve shape which is used as a taxonomic characteristic at the rank of species or variety in this genus. Their habitat is highly variable, from marine and brackish to freshwater. They are generally epiphytic on a red alga *Bostrychia simpliciuscula* under subaerial

conditions, but also sometimes found as an epilithic form on rock and occasionally grow massively to cover an entire rock surface. *Hydrosera triquetra* var. *triquetra* (clone 33-06) and *H. triquetra* var. *hexagona* (clone AH-10) are morphologically distinguishable by their valve outline. The clone 33-06 has a triangular outline with an undulation on each side, giving an impression that the valve has six-polar shape. The clone AH-10 has a genuinely hexagonal symmetry. Our preliminary molecular approach revealed that their ITS rDNA sequences are identical, despite their clear morphological distinctness. This result prompted us to obtain whole genome sequences of both clones in order to reveal the mechanism of shape control by a comparative genomic approach, as well as to discuss about the limit of morphologically defined entities based on their whole genome information. So far the clones 33-06 and AH-10 have been sequenced with Illumina HiSeqX sequencer, and assembled with Platanus 1.2.2, which resulted in total genome size of 31MB (28,053 contigs, N50 = 158 kb) and 23MB (8,132 contigs, N50 = 314 kb), respectively. We will present the whole genomic level comparison which is currently ongoing.

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OPTIMAL CONDITIONS FOR PROMOTING GROWTH AND MATURATION AT THE GAMETOPHYTE STAGE OF UNDARIA PINNATIFIDA BY MULTIVARIABLE ANALYSIS BASED ON INTERACTION EFFECTS

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The objective of this study was to reveal the optimal conditions for promoting the growth and maturation of *Undaria pinnatifida* gametophytes to improve the productivity of seedling production. Four factors were tested: seawater temperature (15°C–25°C), light intensity (2–40 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$), light color (blue: 453 nm, green: 525 nm, red: 641 nm, white: blue + green + red), and day length (0–24 h) in 15 incubators. Free-living gametophytes (male and female) were placed in 24-well microplates with 1/4

PESI medium and were incubated for 4 weeks. All gametophytes were photographed by microscopy once per week and growth rates were calculated by change in area. Maturation was evaluated over 5 phases. The data was analyzed by multivariable analysis and optimal conditions were estimated. The growth of both male and female gametophytes progressed under green light. Optimal seawater temperature and light intensity for the gametophytes were 20.4°C (males), 18.2°C (females), 35.1 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (males), and 37.6 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (females). Maturation of both male and female gametophytes was promoted under blue light, high light intensity, low seawater temperatures, and 14-h light periods. Red light restricted the growth and maturation of gametophytes.

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CYANOBACTERIAL NEUROTOXINS AND THEIR PRODUCERS IN TEMPERATE LAKES IN LITHUANIA

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Neurotoxic cyanobacterial blooms are a worldwide phenomenon, however, in European aquatic ecosystems neurotoxins are less frequently encountered than hepatotoxins. Among neurotoxins, saxitoxins (STXs) are detected more rarely than ANTX-a. In this study, the first report on the presence of STXs and ANTX-a in Lithuanian lakes is represented. The seasonal fluctuation in neurotoxins occurrence was determined in the environmental samples from three eutrophic lakes. In addition, the presence of the toxins, as well as different classes of peptides, was screened in 83 isolated strains of cyanobacteria *Aphanizomenon gracile*, *A. flos-aquae*, *Cuspidothrix issatschenkoi*, *Sphaerospermopsis aphanizomenoides*. STXs were detected in cyanobacterial

samples collected from July to September. The highest intracellular toxin concentrations up to 1.06 µg/l were found in August and strongly correlated with *A. gracile* biovolume values ($r = 0.98$, $p < 0.01$). LC-MS/MS analysis and *sxtA* gene detection confirmed *A. gracile* as being the only and the main STXs producer. Although ANTX-a was detected in the environmental samples (up to 1.90 µg/l in August), none of tested strains was responsible for production. The tested *A. gracile* strains varied with respect to the produced oligopeptides and phycocyanin intergenic spacer (PC-IGS) sequences. They were rich in specific bio-compounds, however just a few strains produced oligopeptides belonging to cyanopeptolins, anabaenopetins and aeruginosins. In conclusion, we identified *A. gracile* as a producer of saxitoxins in Lithuanian lakes. However, analysis of the isolated strains of *A. gracile* with application of chemical and genetic methods showed that the populations are not clonal.

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CHARACTERIZATION AND BIOACTIVITY OF MYCOSPORINE-LIKE AMINO ACIDS EXTRACTS OF THE DIATOMS PHAEODACTYLUM TRICORNUTUM, STAUROSIRELLA PINNATA AND THALASSIOSIRA WEISSFLOGII

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Mycosporine-like amino acids (MAAs) are a class of small, water-soluble molecules known for their capability of absorbing UV radiation. MAAs are widespread across a variety of algae and cyanobacteria. Some red algal MAAs have shown bioactivity, with potential for drug discovery, but there is, as yet, no evidence concerning MAAs production, composition and potential application in cultured diatoms.

In this work two marine diatom strains SAG 1090-1b *Phaeodactylum tricornutum* Bohlin and SAG 122.79 *Thalassiosira weissflogii* (Grunow) G.Fryxell & Hasle and one brackish strain VRUC 290 *Staurosirella pinnata* (Ehrenberg) D.M. Williams & Round were mass cultivated in indoor polyethylene photobioreactors (10 L) to produce biomass for MAAs extraction, performed in methanol/water (20:80 v/v). At first, extracts were characterized using LC-MS, analyzed for their anti-oxidant activity with 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and finally, tested for their bioactivity on human keratinocytes (Ha-Cat) and melanoma cells (CHL-1), preliminary assessment.

Chromatographic profiles indicated that *Staurosirella pinnata* extract had the highest number of MAAs species (7 MAAs identified) including mycosporine-glicine, euhalothece and palitenic acid. *Phaeodactylum tricornutum* and *Thalassiosira weissflogii* chromatograms revealed the presence of shinorine and palithine-treonine-sulfate, for which bioactivity is already documented. This study reports the first experimental evidence and characterization of MAAs in cultures of the these diatoms. *S. pinnata* extract had the greatest anti-oxidant activity with IC50 of 15.0 ± 0.1 mg while *P. tricornutum* and *T. weissflogii* showed values of 75.0 ± 0.2 and 240.0 ± 0.1 mg, respectively.

Thalassiosira weissflogii and *S. pinnata* extract bioactivity was tested on human Ha-Cat and CHL-1 cells. Cytofluorimetric analyses (Fluorescence-Activated Cell Sorting, FACS) indicated that both extracts had a strong cytotoxic effect at the concentrations of 8, 10 and 13.3 mg ml⁻¹, with cell death percentages in the range between 30% to 95% for both cell lines after 24 and 48h treatment.

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SPECIATION OF MACROALGAE IN THE BALTIC SEA

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The formation of new species is an ever-ongoing process, sometimes fast, sometimes slow. Within the Baltic Sea, most of the macroalgae come from a marine origin. They have all entered from the Atlantic waters less than 8,000 years ago, when salty waters flooded into the Baltic basins during the Littorina Sea period. The present salinity gradient, increasing from 1.5 in the north to 8 by the Danish straits in the south, has been stable for the last 4,000 years. The low salinity, which causes osmotic stress, is the most prominent selective force on any marine organism within present day Baltic Sea.

The osmotic stress or salinity selection pressure seems to be hardest on the reproductive phase in the life cycles of macroalgae. It is not uncommon to find a shift towards asexual reproduction in low salinity conditions, such as through tetraspore-to-tetraspore cycling in red algae or by fragmentation. The critical salinity for most gametes sets the sexual reproduction limit around a salinity of 4. Asexual forms of reproduction in low salinities, if frequently occurring, might over time lead to a genetic isolation of groups, divided by direct or indirect effects of salinity gradients.

One such example is the perennial brown macroalgae *Fucus radicans*, which is endemic to and considered to have evolved within the Baltic Sea. It originates from *Fucus vesiculosus*, which has a distinct Baltic Sea ecotype, but *F. radicans* can tolerate lower salinities due to asexual reproduction through fragmentation and re-attachment. Although well adapted to the low salinity of the Baltic Sea, *F. radicans* still contains some of those marine traits that have been lost in the Baltic Sea adapted *F. vesiculosus*. The stable salinity gradient in the marginal system of the Baltic Sea makes it suitable for studying rapid speciation in macroalgae.

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DETERMINATION OF *CHARA*: MORPHOLOGY “VERSUS” GENETICS

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Charophytes are benthic algae with a complex morphology and high phenotypic plasticity. This has led to ambiguities in species delineation. Until recently, genetic studies on *Chara* have been based on samples collected from a restricted geographic range or only included a restricted number of taxa. This may have hindered a general interpretation of the results.

We applied barcoding of *matK*, a rapidly evolving coding section of the plastid genome, in 324 *Chara* samples collected from 19 countries, in order to test whether the distribution of barcode haplotypes among individuals was consistent with species boundaries as they are currently understood.

The phylogenetic tree grouped the 324 *Chara* individuals, which according to commonly used identification keys represented 29 species, into 12 well-defined groups (i.e. monophyletic morphospecies or groups of morphospecies). Considerable morphological variation occurred

within genetically homogeneous groups. This included traits which are commonly used for *Chara* species determination, such as the length and number of spine cells, the length of stipulodes and bract cells, cortication (tylacanthous, isostichous, aulacanthous and absent cortication), as well as sex differentiation. However, there were also substantial genetic differences among morphologically similar species (e.g. *C. virgata* – *C. globularis* – *C. connivens*). No morphological trait consistently reflected genetic differences. This indicates that morphological traits for specific taxa may serve as diagnostic tools for species delimitation, but that they are not generally suitable for inferring genetic differentiation or phylogenetic relationships.

Preliminary results on the charophyte genus *Tolypella* point in the same direction. We found cryptic species (there occur two genetically distinct groups of *T. glomerata*), as well as morphological variation within genetically homogeneous groups (e.g. *T. normaniana* – one group of *T. glomerata*).

Many charophyte species are red listed, and our findings may have impli

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FUNCTION AND DIVERSITY OF THE EPIPHYTIC BACTERIAL COMMUNITY ON THE ARCTIC MAERL, LITHOTHAMNION GLACIALE

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In the marine environment, all exposed substrates are readily fouled. Because algae ‘leak’ exopolymeric substances, specific bacterial groups are hypothesized to preferentially recruit to algal surfaces, forming distinct biofilm communities. This association reciprocally influences the ecology, physiology, and biochemistry of both bacteria and algae. For instance, the bacteria in the biofilm probably gain nutritional benefits from algae, and concomitantly influence the physiochemical environment of the host alga by acting as a diffusive boundary layer between the alga and its environment. However, biofilm composition as well as metabolite production by the algal host or sympatric species can be influenced by geographic location and characteristics of the physical environment (i.e. light and temperature). Given the susceptibility of polar near-shore marine habitats to elevated stressors due to climate change, this study set out to quantify the bacterial diversity in maerl biofilms from two locations in the fjord-marine confluence near Nuuk,

southwestern Greenland. Furthermore, we tested the stability of biofilm composition under fluctuating temperature and salinity gradients (4–7°C, 22–33 psu) that reflect conditions of high ice sheet melt. We identify taxonomic and inferred functional diversity of biofilms within the water column, innate substrate, and on maerl in the field and in mesocosm treatments. This allows us to look at potential shifts in these important biofilm communities under variations in their environment and by including inferred function of certain members we can gain insight into effects on the host maerl.

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PROGRESS IN IRISH KELP FOREST ECOLOGY: INSIGHTS TO PRODUCTIVITY AND BIODIVERSITY OF LAMINARIA HYPERBOREA FORESTS

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The population structure and productivity of dominant kelps in the subtidal habitat of western Ireland is currently understudied. Some recent investigations highlight the productivity of common seaweeds in a range of environmental parameters and the biodiversity of kelp holdfast habitats. However, little is known about processes within subtidal kelp forests dominated by *Laminaria hyperborea*. This project will create a baseline for the structure and productivity of natural kelp forests in this region as a reference for future studies. Initial surveys of kelp forests at four permanent sites on the west coast show variability in species diversity and abundance across the granite (Co. Galway) and limestone (Co. Clare) habitats in winter. Winter surveys indicate that *L. hyperborea* reaches a maximum age of 8–9 years within the kelp canopy, fosters other kelp species and <1 year recruits in varied abundance, and represents approximately 5 kg algal biomass m⁻² in the winter-spring. Preliminary summer measurements show higher kelp biomass, growth of juvenile individuals, and warmer temperatures and higher light conditions. Further seasonal measurements of biodiversity, population structure and productivity, recruitment-succession, and environmental parameters within this habitat will be useful in assessing the standing stock of *L. hyperborea* and ecological status. Year-round monitoring in specific locations will be supplemented by surveys completed in partnership with recreational divers in Ireland,

broadening the focus on algal dominated communities in this program.

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LINKING PHENOTYPE WITH GENOTYPE IN THE BENTHIC DIATOM SEMINAVIS ROBUSTA

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Reduced gene flow between populations is considered a prerequisite for the development of genetic differentiation, local adaptation and speciation. In microscopic organisms, such as microalgae, geographic distance, heterogeneous environmental conditions or historical events have been most frequently proposed as barriers to gene flow. However, in the benthic pennate diatom *Seminavis robusta* we find the coexistence of three genetically differentiated groups (mating groups), between which mating is drastically reduced. Because these mating groups coexist in both time and space, geography and history are less likely mechanisms driving reduced gene flow. Although crossing between mating groups is possible in the lab, it is still less successful than crossing within mating groups, and genetic information supports that under natural conditions interbreeding is a less common event. Here we present phenotypic characterization of 48 strains of *S. robusta* for which the genomes have been sequenced, focusing on environmentally important traits (e.g. temperature optimum, light and nutrient requirements). This allows us to explore additional causes and/or consequences to the reproductive barriers observed between these coexisting mating groups.

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FUNCTIONAL ANALYSES OF SEX SPECIFIC RECEPTOR-LIKE PROTEINS IN CLOSTERIUM PERACEROSUM-STRIGOSUM-LITTORALE COMPLEX, USING CRISPR/CAS9 SYSTEM

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Heterothallic strains of *Closterium peracerosum-strigosum-littorale* complex (*C. psl.* complex) have two sexes, mating type plus (mt+) and mating type minus (mt-). The progress of the conjugation is regulated by two sex pheromones, PR-IP and PR-IP Inducer, which are produced by mt+ cells and by mt- cells, respectively.

To characterize the sexual reproduction processes, at first, we focused on the *CpRLP1* gene, encoding a receptor-like protein and expressing in mt- cells in response to PR-IP. We constructed a vector for CRISPR/Cas9 using an endogenous *CpU6* gene promoter and a codon-optimized *Cas9* gene and established several *CpRLP1*-knockout mt- strains. When the transformants were mixed with wild mt+ cells, conjugating reaction was severely reduced, compared to the controls. Many cells released their protoplasts without pairing, suggesting the loss of synchronization between two mating partners. Furthermore, transformants showed a hyper sensitivity to the PR-IP.

Next, we characterized *CpRLK2* gene. It encodes a receptor-like protein kinase, in which the extracellular domain showed 40% identity to that of *CpRLP1*. It was expressed in sexually competent mt- cells, differentiated from vegetative cells after the appropriate culture. In contrast to the *CpRLP1*, the expression of *CpRLK2* was not stimulated by the addition of PR-IP. The knockout transformants did not show the mating reaction and did not respond to the PR-IP. From these results, we consider that the *CpRLK2* is a possible receptor candidate for the PR-IP and the *CpRLP1* functions as a down-regulator of PR-IP response, which is indispensable for the progress of the conjugation of *C. psl.* complex.

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CYSTOSEIRA MONTAGNEI J.G. AGARDH AND C. SPINOSA SAUVAGEAU (PHAEOPHYCEAE, SARGASSACEAE): A TAXONOMIC REAPPRAISAL OF MISUSED NAMES, WITH THE PROPOSAL OF CYSTOSEIRA MICHAELAE VERLAQUE ET AL. NOM. NOV

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Cystoseira granulata C. Agardh var. *turneri* Montagne was described by Montagne (1838) from Algeria (Southern Mediterranean Sea). Subsequently, J.G. Agardh (1842) re-named the taxon as *C. montagnei* J. Agardh, on the basis of specimens from France and the northern Adriatic (northern Mediterranean Sea) he believed identical to Montagne's taxon. Finally, Sauvageau (1912) described *C. spinosa* Sauvageau and *C. adriatica* Sauvageau as nomina nova, partly to accommodate Agardh and some other authors' concept of *C. montagnei*. These taxonomic treatments caused confusion regarding the delineation of these taxa and doubts have been raised regarding the taxonomic value of Montagne's taxon, which today is often listed as *taxon inquirendum* in updated checklists and floras. We have collected Montagne's taxon (here *Cystoseira michaelae* Verlaque et al., nom. nov.) since 2014 near Algiers (Algeria) where it forms sparse forests between 10 and 25 m depth. Our collected specimens of *C. michaelae* match the original description as well as the original material of Montagne's taxon. They are well characterized in having a single axis with young tophules spinose becoming smooth-tuberculate when older, primary branches either slightly compressed with an inconspicuous rib and irregularly alternate in one plane, or cylindrical and branched in all directions, with spaced short spine-like appendages, and conceptacles both intercalary basal, just above the tophule, and terminal on branchlets. Here, we propose *Cystoseira michaelae* Verlaque et al. nom. nov. to accommodate Montagne's taxon (*Cystoseira granulata* C. Agardh var. *turneri* Montagne) and the typification of the species on the basis of both Montagne's protologue and of an original specimen of Montagne's housed at the Muséum National d'Histoire Naturelle de Paris (PC).

SEASONAL VARIATION OF BACTERIAL COMMUNITIES ASSOCIATED WITH THE INVASIVE BROWN SEAWEED *SARGASSUM MUTICUM*

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Seaweed-associated microbiota show small and large scale spatial differences linked to environmental conditions and seaweed physiology. To what extent these differences are seasonal is hardly studied. Shifts in microbial abundance and composition, in response to seasonally changing environmental parameters, may result in structural, functional and behavioral changes within the host with potential consequences for its fitness, and thus on its ability to acclimatize to changing environmental conditions. In this study, we used high-throughput sequencing of 16S rRNA to explore the seasonal variation of bacterial communities associated with the invasive brown seaweed *S. muticum* (across different tissue types) in northern and southern Portugal, in September, March, and July-August (summer). The bacterial diversity was higher in the North as compared with the South for geographical locations, and on basal parts as compared with apical parts for tissues. Bacterial community composition in summer was very distinct from September and March, independent of the location of the study site, mainly due to a strong decrease in *Proteobacteria* (Non-ID Rhodobacteraceae and *Loktanella*) and *Actinobacteria* (*Acidimicrobiia*), as well as an increase in *Planctomycetes* (Non-ID *Pirellulaceae*). Core bacteriomes were very similar between North and South Portugal in September and March, but not so in summer. At both locations, seasonal differences were most pronounced in the apical tips and sediments, but the bacteria contributing to these differences differed between locations. Small and large scale spatial differences in associated bacterial communities were most pronounced in summer and apical tissues. We observed a successional pattern in the seaweed-associated bacterial communities with a gradual addition of pathogenic bacteria towards summer. These differences have the potential to affect seaweed ecology, fitness and adaptation capacity to environmental changes.

WANTED: DEAD AND ALIVE! INTEGRATING EXTANT WITH EXTINCT TO TRANSFORM OUR UNDERSTANDING OF COCCOLITHOPHORE PHYSIOLOGY, ECOLOGY AND EVOLUTION

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The extensive fossil record of coccolithophores, a major calcifying algal group, is a 200-million-year archive of adaptive responses of entire communities to gradual and rapid climate change. However, the measures of fitness routinely used by phycologists, including growth, photosynthetic rates, calcification rates and cell size, are elusive to micropaleontologists as only the mineralized hard parts (exoskeletal coccosphere) are preserved, typically disaggregated into component parts (coccoliths).

The presence of intact fossil coccolithophore ‘cells’ in exceptionally well-preserved sediments bridges this divide by providing new tools with which to investigate the paleo-physiology, paleo-ecology and evolution of ancient species. Across >4,300 individual fossil coccospheres, we observe diverse intra- and interspecies architectures and identify specific geometric relationships, linking cell size, coccolith size and coccolith numbers. To understand the role of physiology in coccosphere geometry we carried out laboratory experiments on long-lived extant species, identifying cell size and coccolith number as traits responsive to growth phase. This provides a new proxy for growth phase, where greater proportions of smaller cells with fewer coccoliths characterize phases of rapid cell division.

By integrating phycology and micropaleontology using this growth proxy, coccosphere data and cell size models, we can investigate coccolithophore paleo-records across major climate transitions, including rapid warming events (the Paleocene-Eocene thermal maximum, 56 million years ago) and the switch from warm, greenhouse climates to cool, icehouse climates 44 to 32 million years ago. We find significant expansions in community cell size that reflect interactions between environmental conditions, physiology and evolutionary strategies. We also see environmentally-forced shifts in biomass and calcite production

reflecting changes in growth rate, coccosphere geometry and community composition. Combining phylogenetic approaches with new coccosphere-based data from extant and extinct species therefore provides a unique perspective on spatio-temporal trends in physiology, ecology and species-success at cellular, population and community levels across a range

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SEX-SPECIFIC EXPRESSION OF IMPORTIN ALPHA HOMOLOGUES DURING FERTILIZATION IN A RED ALGA, *BOSTRYCHIA MORITZIANA*

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Differential expression of sex-related genes was analyzed using RNAseq based on pyrosequencing of male and female gametophytes of *Bostrychia moritziana*. Two homologues of importin alpha which are involved in protein transport into the nucleus were isolated from the transcriptomes and named as BmFS1 and BmMS1. Conserved domains of ARM repeat and Ankyrin repeat were found in both genes, but the sequence identity was less than 77%. BmFS1 and BmMS1 were sex-specifically expressed in the female and male gametophyte, respectively. Both genes were expressed in the tetrasporophyte and carposporophyte. Genomic PCR confirmed that BmFS1 and BmMS1 exist only in the female and male gametophyte, respectively. Expression of these sex-specific importin alpha genes was observed using qPCR during fertilization. The expression of female-specific importin alpha (BmFS1) sharply increased as soon as male spermatia attached to the female trichogyne, and gradually decreased over the time-course. Our results indicate that sex in *Bostrychia moritziana* is determined by sex chromosomes, and female-specific importin alpha may be involved in protein traffic to the nucleus during the fertilization process.

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FORMATION AND GERMINATION OF TEMPORARY CYSTS OF *COCHLODINIUM POLYKRIKOIDES* MARGALEF (DINOPHYCEAE) AND THEIR ECOLOGICAL ROLE IN DENSE BLOOMS

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While the initiation and development of dense bloom of *Cochlodinium polykrikoides* have been shown to be related to some environmental factors, little is known about the ecological role of the formation and germination of temporary cysts, nor of their significance for the rapid expansion of dense regional-scale blooms. This study examined the factors affecting the formation and germination of temporary cysts of *C. polykrikoides*, and provides details about the germination process. In the laboratory experiments, *C. polykrikoides* produced chain-forming temporary cysts which are immobile and surrounded by a hyaline membrane. The encystment experiment indicated that darkness induces the formation of chain-forming temporary cysts, consistent with field observation of morphology and fluxes of temporary cysts. Germination occurred twice from a single four-celled temporary cysts within 24 h of exposure to light, and the germlings appeared as two-celled chain-forming vegetative cells. The germination behavior of temporary cysts of *C. polykrikoides* differs from that of other dinoflagellates and this may be a survival strategy for the maintenance of population size during dense blooms.

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PINPOINTING THE ACQUISITION OF COMPLEX RED ALGAL PLASTIDS IN CRYPTOPHYTA – A PHYLOGENOMIC INVESTIGATION OF THE CRYPTOMONAD *GONIOMONAS AVONLEA*

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Cryptomonads are eukaryotes comprised of both photosynthetic and non-photosynthetic species. While cryptomonads such as *Guillardia theta* harbor a red-algal plastid of secondary endosymbiotic origin, members of Goniomonadea, including *Goniomonas avonlea*, lack plastids entirely. A long-standing question in the field of plastid evolution is whether the Goniomonadea are ancestrally non-photosynthetic or whether they lost a plastid secondarily. To address this, we have sequenced the genome and transcriptome of *G. avonlea* and performed phylogenomic analyses to search for possible footprints of an algal endosymbiont. Overall, there is little evidence for a significant contribution of red-algal genes to the nucleus of *G. avonlea*. We nevertheless found a large proportion of genes showing a relationship to green algae. Whether these genes should be interpreted as evidence for a plastid in a common ancestor shared by *G. theta* and *G. avonlea*, or reflect deep host affiliations between cryptomonads and Archaeplastida is uncertain. A plastid-bearing ancestor of Goniomonadea and cryptophytes has important implications for hypotheses of plastid evolution and answering this question will help to understand the evolution of plastids in Cryptophyta and other complex plastid-bearing algae. Additionally, the *G. avonlea* genome provides an important reference point for elucidating how secondary plastid acquisition in *G. theta* and other photosynthetic cryptomonads subsequently altered their genomes and proteomes.

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GIANT SCALES, TINY SCALES & SIMILAR SCALES: A REVIEW OF THE SYNUROPHYCEAE IN THE FOSSIL RECORD

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Synurophyceae are heterokont algae characterized by distinctive siliceous scales that cover the surface of the cell, and are ecologically important in inland waters. The evolutionary history of the group is poorly known largely due to a sparse fossil record. Recently, scales representing over 55 synurophyte species have been uncovered from four

geologic localities representing the Paleocene (Wombat, n = 10), Eocene (Giraffe and Horsefly, n = 45) and Miocene (Virgin Valley, n = 3). The vast majority of fossils have come from the Giraffe fossil locality, a maar lake that existed in the Canadian Arctic during the middle Eocene under a warm greenhouse climate. We combined a five-gene molecular phylogeny with the fossil remains to explore evolutionary trends within the group, with a focus on the siliceous components. Based on these results, the group originated in the Jurassic approximately 157 million years ago (Ma), with the keystone genera *Mallomonas* and *Synura* diverging during the Early Cretaceous at 130 Ma. *Mallomonas* further splits into two major subclades, signaling the evolution of the V-rib believed to aid in the spacing and organization of scales on the cell covering. *Synura* also diverges into two primary subclades, separating taxa with forward-projecting spines on the scale from those with a keel positioned on the scale proper. Fossil representatives from all major subclades have now been uncovered from the early Cenozoic. Many of the fossil species that are extinct and lack modern congeners had gigantic scales, and based on a model relating scale size to cell size, they also had large cells. The majority of extinct taxa also had robust bristles, and their scales lacked V-ribs and well-developed domes indicating that these characters are probably ancestral. Scales of other fossil species are virtually identical to modern forms, resulting in evolutionary stasis in scale design extending over tens of millions of years.

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IDENTIFYING PROTEINS IMPORTANT FOR COCCOLITH FORMATION IN EMILIANIA HUXLEYI

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Species such as *Emiliana huxleyi* form large blooms in the oceans which have important ecological and biogeochemical consequences. In addition, the intricate patterning of coccoliths on a nano-scale makes them attractive for nanotechnological applications. Thus a detailed understanding of the molecular mechanisms of calcification is desirable. Although some ion channels putatively involved in

calcification have been identified from the *E. huxleyi* genome, our knowledge of the protein machinery involved in processes such as control of calcite crystal morphology, the development of the coccolith vesicle or the regulation of calcification is still very limited. To identify proteins involved in calcification in *E. huxleyi* we took two main approaches. The first was a focused strategy, analysing the proteome of extracellular coccoliths. The second approach took a wider, whole-cell perspective, and involved a detailed quantitative proteomic time-course of cells re-calcifying after initial growth in non-calcifying conditions. In parallel we analysed the newly-synthesised proteome of these cells, so as to identify proteins that changed in turnover rate if not in abundance. To generate good quality data we had to solve a number of challenges, including generating a new predicted proteome for *E. huxleyi*. This was necessary because the gene models associate with the *E. huxleyi* genome are largely *ab initio* predicted using algorithms trained on very distantly related species. Thus it seemed likely that although many gene models encoding highly conserved proteins would be accurately predicted, those genes encoding proteins involved in taxon-specific functions such as calcification would be less well predicted. Analysis of our mass spectrometry data supported this supposition. To generate better gene models we used long-read cDNA sequencing to generate a catalogue of transcript sequences and their isoforms. The improvement this made to our proteomic analyses will be presented, along with the properties of the proteins identified and their relevance for our understanding of calcification.

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DNA BARCODING FOR ASSESSING DISTRIBUTION PATTERNS IN CRYPTIC SEAWEED SPECIES: THE 'CODIUM' CASE STUDY IN THE BAY OF BISCAY, NORTHEAST ATLANTIC OCEAN

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The infamous seaweed invader *Codium fragile* ssp. *fragile*, distributed on rocky shores worldwide, is officially listed as one of the 100 worst invading species in Europe. As it is prone to crypsis, the application of genetic molecular data is needed to support species identification. *Codium* spp. are recorded in a checklist of the benthic flora in the Cantabrian Sea (North of Spain) but detailed biogeographical distributions, origins, ecological mechanisms explaining their successful establishment and genetic structuring data from existing species are scarce. In this work, the main aim was to assess the *Codium* species distribution along the central southern area of the Bay of Biscay, supporting these data with DNA – based identifications using sequencing of the RuBisCO and plastids elongation factor TU (*tufA*) genes. Initial results showed that three different species of *Codium*: the invasive *Codium fragile* ssp. *fragile* and the native *Codium vermilara* and *Codium tomentosum* were found cohabiting this area, but they have different seasonal and geographic patterns. More samples from the Portuguese and Spanish Atlantic areas are currently being included in this study to extend the scope of the results and conclusions.

This is the first extensive genetic and distribution study on cryptic species from the genus *Codium* in the Bay of Biscay, northeast Atlantic Ocean, and it is significant not only for assessing species distributions, but also morphology-environment-genetics interactions. The primary results indicate differences in a winter and summer sampling of invasive *Codium fragile* ssp. *fragile* and native *Codium* species along Cantabrian coast. This knowledge will help to identify relevant key points for the prevention and management of seaweed biological invasions.

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NITELLOPSIS OBTUSA IN NORTH AMERICA

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The charophytic green alga *Nitellopsis obtusa* (Characeae) was first reported in the New World in the St. Lawrence River, NY, U.S.A. in 1978. Since that time, *N. obtusa* has been widely reported from inland lakes throughout northern Indiana, Michigan, and western New York, with isolated locations in Indiana, Minnesota, Pennsylvania, Vermont, and Wisconsin U.S.A., as well as Ontario and Québec, Canada. *Nitellopsis obtusa* has been identified as a threat to native ecosystems and recreational activities

and is listed as an aggressive invasive species by the United States Geological Survey. The vector(s) by which *N. obtusa* was transported to North America and distributed across the landscape remain unknown. In 2015 and 2016, 740 water bodies were surveyed for *N. obtusa* across New York and New England. Results from this work suggested that *N. obtusa* is primarily moved via recreational boating. However, in other states, birds have been hypothesized to transport *N. obtusa*. Organellar genome sequencing and Genotyping by Sequencing (GBS) methods use next-generation sequencing to identify single nucleotide polymorphisms (SNPs) from across a genome. The quantity and distribution of SNPs generated by these methods are ideal to address population-level questions about movement into, and distribution across, the landscape. Analysis of mitochondrial and plastid genomes identified variation between populations in Asia, Europe, and North America. However, little variation was detected among North American organellar genomes, indicating that clonal spread of a single introduced population is possible. Results from GBS data revealed a complicated pattern of spread across North America and suggest that increased sampling from within the invaded range is needed to better understand the spread of *N. obtusa*.

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USING SPECIES DISTRIBUTION MODELS TO EXAMINE THE FUTURE OF CHARACEAE IN NORTH AMERICA

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Freshwater ecosystems are rich in biodiversity, economically important, and increasingly impacted by human activities. Aquatic macrophytes are vital components of freshwater ecosystems that provide numerous ecosystem services. The Characeae, or stoneworts, are a family of green macroalgae found in aquatic ecosystems on all continents except Antarctica. Species in this family are integral parts of ecosystems: they provide forage for birds, invertebrates and fish, and are important for colonizing new habitats and stabilizing sediments. A clear understanding of the distributions and species–habitat associations of aquatic macrophytes is essential for predicting the responses of aquatic systems to global and regional change. Species distribution models can be used to gain insight into ecological

processes and allow prediction of distributions under future environmental scenarios. This study uses a survey of 740 water bodies, 11 chemistry variables and 55 climate variables to build species distribution models for 10 species of Characeae across the Northeast USA (an area greater than 325,000 square kilometers). The relative importance of predictor variables was assessed by building models with these chemistry and climate data separately and combined. Models built using chemistry variables alone generally outperformed those built with climate variables. The effects of eutrophication, climate change, and cation leaching on distributions of Characeae species were quantified, with some species expected to increase their range, and others predicted to lose all suitable habitat. We demonstrate that Characeae species have distinct habitat preferences, with some species predicted to be more sensitive to environmental change than others, and argue that landscape scale conservation must account for differences in habitat to conserve these ecologically important macrophytes.

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MICROALGAE OF SAND SUBSTRATES OF THE NORTH-WESTERN BLACK SEA COAST

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The sandy supralittoral (psammocontour) is area of great importance for coastal ecosystem because of its participation in productive processes, transferring elements through the food chain as well as removal of nutrients and organic matter. The objective of this study was to understand the peculiarities of formation of phytosammic community of the north-western part of the Black Sea. We analyzed the influence of 14 environmental parameters on microalgae of the splash zone. The priority factor for psammon algal community in any season is size of sand grains and siliceous oxide. Other variables depend on the season: while in summer, mineral nitrogen (nitrates and nitrites) effects the microphytes more intensively than others, in autumn it is replaced by organic nitrogen and silty fraction. The effect of size of sand particles was studied in the course of laboratory experiment using specially designed plates. It was shown that a greater abundance of microalgae registered on the grains with a smaller particle diameter (<0.25; 0.25–0.50 mm). The experimental data allow us to predict the number of microalgae in a changing particle size distribution of the sand. In the phytosammon 100 taxa of algae were found: 78 Bacillariophyta, 4 Chlorophyta,

4 Euglenophyta, 2 Cryptophyta, 3 Dinophyta and 9 Cyanoprokaryota. We registered 8 new species for the north western Black Sea coast: *Attheya decora* T. West, *Halamphora salinicola* Levkov et Díaz, *Diploneis stroemii* Hust., *Navicula viminoides* var. *cosmomarina* Lange-Bert., A. Witkowski, Bogaczewicz-Adamczak & Zgrundo, *Navicula phylleptosoma* Lange-Bert., *Opephora minuta* (A. Cleve) A. Witkowski, *Dinema validum* Larsen et Patterson and *Heteronema larseni* Lee et Patterson.

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ROLE OF PHYTOPLANKTON METABOLISM DOWN-REGULATION IN A HIGH CO₂ WORLD

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The oceans play a major role as a sink for up to 26% of the anthropogenic CO₂ produced by emission from fossil-fuel burning, cement production, deforestation and other land-use changes. Most marine phytoplankton species have active Carbon Concentrating Mechanisms (CCMs) under present CO₂ concentrations. But down-regulation of CCMs activity is expected for high CO₂ levels predicted for future climate scenarios compared to present conditions. In this study we present the results from 6 different marine phytoplankton species acclimated during at least three weeks to high CO₂ concentrations predicted for future scenarios of global change (1000 ppmv de CO₂, High Carbon: HC) and to present CO₂ concentrations (380 ppmv CO₂, Low Carbon: LC). Metabolic rates, CCM expression and concentration of pigments and polyunsaturated aldehydes were measured in HC and LC cultures under two different metabolic conditions: in CO₂ acclimated cells with steady metabolic conditions (i.e. after at least three weeks CO₂ acclimation) and in cells with perturbed metabolism due to the detention of the aeration during 4 h. The results show that down-regulation triggers many reactions in the cell with significant decreases in the intracellular pools and the enzymatic activities, that increases the resource use efficiency, finally resulting in similar or higher photosynthetic and growth rates than under present levels of CO₂. However,

the opposite was observed after perturbing the cultures and promoting the up-regulation of the metabolism. The study demonstrates that phytoplankton production and growth response to ocean acidification changes with cell metabolism and depends on the energetic cost of metabolite synthesis and activity.

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THE GENUS EUNOTIA EHRENBERG (BACILLARIOPHYCEAE) IN TWO HIGH ALTITUDE LAKES IN BLACK SEA, TURKEY

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The genus *Eunotia*, belonging to the family Eunotiaceae, was firstly described by Ehrenberg in 1837. Among these freshwater diatoms, the genus is represented over 500 species, usually it is a common genus in freshwater habitats and mostly found in oligotrophic and acidic streams. The Black Sea region, has high mountains that run parallel to the shoreline and which include many high altitude lakes (>3,000 m) in the eastern part of the region and springs are mostly isolated in the mountains. In this study, the samples were collected from two high altitude lakes (about 3,400 m) and some small streams flowing into the lakes in July 2015. *Eunotia* species are good tools to understand the distinct habitats of the lakes in the region. As a result, 11 *Eunotia* taxa have been found. *E. bilunaris* (Ehrenberg) Schaarschmidt is common in different river catchments; *E. valida* Hustedt is common in some other lakes in the same region and 7 species (*E. boreoalpina* Lange-Bertalot & Nörpel-Schempp, *E. botuliformis* F. Wild, Nörpel

& Lange-Bertalot, *E. crista-galli* Cleve, *E. curtagrunowii* Nörpel-Schempp & Lange-Bertalot, *E. islandica* Østrup, *E. minor* (Kützing) Grunow, and *E. subarcuotoides* Alles, Nörpel & Lange-Bertalot) are new records for the “Fresh-water Flora of Turkey”.

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DIATOM ASSEMBLAGES FROM SIMILAR PHYTOGEOGRAPHICAL REGIONS IN TURKEY AND IRAN

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As neighbors, Turkey and Iran have similarities and differences in the composition of their biodiversity. From a biogeographic point of view, Turkey is comprised of three main phytogeographical regions (Euro-Siberian, Irano-Turanian and Mediterranean). This diversity is due to variability in geologic and climatic conditions across the country. The province of Kütahya is an ecotone between these phytogeographical regions. Iran is also a large country with three phytogeographical regions (Irano-Turanian, Euro-Siberian and Saharo-Sindian elements). The Albourz Mountains lie in northern Iran and are affected by Euro-Siberian and Irano-Turanian climatical conditions, suggesting some similarities with Kütahya in Turkey.

In this study, the diatom flora of some running waters in Kütahya, Turkey, and the Karaj River in the Albourz Mountains, Iran, were compared. The most common diatoms in Kütahya running waters were *Achnanthydium bisolettianum* (Grunow) Bukht., *Amphora pediculus* (Kützing) Grunow ex A.F. Schmidt, *Cymbella excisa* Krammer, *Cymbopleura amphicephala* (Nägeli) Krammer and *Diatoma moniliformis* (Kützing) D.M. Williams, whereas in the

Karaj River *Amphora indistincta* Levkov, *Diatoma maxima* (Grunow) Fricke in A.Schmidt, *Encyonopsis microcephala* (Grunow) Krammer and *Surirella minuta* Brébisson ex Kützing were the most common taxa.

However, some diatoms were common between these study areas, including *Adlafia minuscula* (Grunow) Lange-Bertalot & Genkal, *Amphora inariensis* Krammer, *Amphora ovalis* (Kützing) Kützing, *Caloneis lancettula* (Schulz) Lange-Bertalot & Witkowski, *Denticula tenuis* Kützing, *Frustulia vulgaris* (Thwaites) De Toni, *Navicula antonii* Lange-Bertalot, and *N. reichardtiana* Lange-Bertalot. While these species are widely distributed, this marks a first step in comparing the freshwater diatoms from rivers in these understudied regions.

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ALGINATE BEADS: A STUDY OF BEAD STABILITY AND CR (VI) REMOVAL

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Alginate has been considered recently as a microorganism immobilized matrix and biosorbent of cations. In order to find a stable structure to remove Cr (VI) from synthetic wastewater, we compared different concentrations and also combinations of alginate with other polymers and salts at room temperature and 70°C. Chitosan (0.1–1%), hydroxyl methyl cellulose (1–2.5%) and PVP (1–20%) beside NaCl (1%) or CaCO₃ (1–10%) were added to alginate (1–3%) then solidified in BaCl₂ or CaCl₂ (2%). *Scenedesmus quadricauda* (1–4 g/L) was added to the alginate suspension to increase biosorption capacity. Based on bead resistance in phosphate solution (0.1–1 M) during 4 days, more stable beads were selected then Cr (VI) removal by algal and alginate beads was studied. Results showed beads including 2% alginate (without any other polymer) in combination with 1% CaCO₃ that had been titrated in BaCl₂ made more resistant and biosorbent beads in the presence of phosphate and Cr (VI), respectively. Ba formed alginate beads firmer than Ca but with a decrease in *S. quadricauda*

**CONTRIBUTION TOWARDS
MORPHOLOGICAL AND MOLECULAR
TAXONOMIC REFERENCE LIBRARY
OF BENTHIC MARINE DIATOMS FROM TWO
ARCTIC FJORDS ON SVALBARD (NORWAY)**

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Diatoms play an important role in the oceans, not only in the planktonic realm but also in the benthic habitat, where they contribute in high abundance to the microphytobenthos. Until recently, only a few investigations existed on the occurrence and distribution of marine benthic diatoms in Antarctica and the Arctic. Although knowledge on benthic diatom taxonomy and hence biodiversity from Arctic locations is rare, the few existing analyses address morphological characteristics only. The main goal of our study was for the first time to combine both morphological features and DNA information on benthic Arctic diatoms. We studied the morphology of 26 strains from the Rostock Culture Collection of Arctic diatoms via light and electron microscopy. We addressed their molecular taxonomy by sequencing the DNA barcode 18S V4 and the chloroplast marker *rbcL*. As a result, 10 raphid, one araphid, and one bipolar centric diatom taxa are presented in this study. From these, three species were described as new to science. Although the studied strains represent only a minor fraction of the diatom flora of Svalbard, this study is a first milestone in coupling morphological and molecular data of benthic diatoms in such high latitudes. By making these data available online, they contribute to a taxonomic reference library for Arctic benthic diatoms and are available for comparative studies. Consequently, the morphological and molecular taxonomy of these ecologically important microalgae has to be urgently addressed to prepare a reliable floristic list

population in the beads. Although beads with PVP coverage showed positive effects in high pH, other polymers didn't change the stability. In addition, application of all polymers in the alginate beads structure reduced Cr (VI) removal whilst *S. quadricauda* enhanced it by up to 15%. The highest removal rate for hydroxyl methyl cellulose coverage beads (24%) happened within 2 h while for other beads it was in the first 6 h (37%). It was suggested to stop removal after 24 h since saturation stage happened after that (52%). Accordingly, use of fresh beads which has been polymerized in 70°C for 1 h and kept in solidified solution for 4 h, improved Cr reduction up to 86% during 24 h. Results from this study suggest that algal alginate beads are an effective sorbent for Cr (VI) removal from aqueous solutions and for biofilter design.

MEIOSIS IN PORPHYRA UMBILICALIS

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Meiosis, an essential step for sexual reproduction, is crucial for maintaining genetic diversity and is widespread in eukaryotic lineages. Sexual organisms are often determined through the presence or absence of necessary meiotic genes in a genome. The red algae, *Porphyra umbilicalis*, which inhabits the northeastern United States is believed to be asexual. To find molecular evidence to support this notion, we analyzed the genome of this strain of *P. umbilicalis* and looked for meiosis genes. Six (SPO11, HOP1, HOP2, MND1, MSH4, MSH5) of the nine (DMC1, REC8, MER3) most common eukaryotic meiosis-specific genes were found. In addition, *P. umbilicalis* appears to lack five other genes found in several eukaryotes that simultaneously encompass roles in DNA repair, mitosis, and meiosis: MLH2, MLH3, PDS5, RAD21, and SSC3. However, the significance of these absences requires further study, as some of these genes are not present in clearly sexual organisms and it is possible that other proteins support these functions in this *P. umbilicalis* strain. For future work, we propose to further investigate the missing genes and compare the genetic composition of *P. umbilicalis* to a clearly sexual organism.

and thus, proper knowledge on their biodiversity. Such data would then represent the baseline for any future monitoring approaches to document changes in structure and function of the benthic diatom flora of Svalbard. Since we still lack baseline biodiversity data on benthic diatoms in Kongsfjorden and other Arctic marine ecosystems, it is very difficult to interpret community changes, for example, due to global warming.

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**SOME PRELIMINARY STUDIES ON TAXA
IN THE GENUS GRAMMONEMA
(BACILLARIOPHYTA) USING HERBARIUM
MATERIAL FROM THE DIATOM
COLLECTION OF THE NATURAL HISTORY
MUSEUM, LONDON, UK**

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The marine genus *Grammonema* Agardh (= *Grammatonema* Kützing) is found in areas that are critical for documenting climate change in polar waters. There are six names in the genus but it is not certain how many of these represent diagnosable taxa. To provide new taxonomic/biodiversity data, herbarium material housed in the diatom collection at the Natural History Museum, London, was examined. This material was originally preserved dried directly onto pieces of mica. After processing the material, this allows examination using scanning electron microscopy of original type specimens.

In this study, we present our preliminary observations on some type specimens preserved in this way from early work of Greville and Ralfs:

- (1) *Grammonema striatulum* var. *diatomoides* (Greville) Kützing (1849) = *Grammonema juergensii* var. *diatomoides* (Greville in Harvey) Rabenhorst (1864) = *Fragilaria diatomoides* Greville in Harvey (1833); and
- (2) *Grammonema juergensii* [var. *alpha*] Ralfs 1846.

In this study we will review the morphological data gained from examining these specimens, present some details on the taxonomy of the group and show how they offer further support for biogeographical studies on marine genera occurring in areas sensitive to global warming of polar zones.

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**CONSPECIFICITY OF AN INVASIVE
ECOTYPE OF *ULVA COMPRESSA*
FROM THE SW BALTIC WITH THE MODEL
ORGANISM *ULVA MUTABILIS***

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The genus *Ulva* has broadly negative connotations because of its ability to form harmful “green tides” and the problems it causes with precise species identification, due to its morphological plasticity. During recent years, tides of unattached *Ulva compressa* U. Linneus 1753 with an atypical sheet-like morphology were for the first time observed in the German Baltic. Here we report that this nuisance alga is conspecific with the type strain of *U. mutabilis* Föyn 1958 from Faro in Portugal, an important model organism to study morphogenesis, morphogenetics and mutualistic interactions. Different approaches were used to examine conspecificity: (1) Comparisons on vegetative and reproductive features of cultured material of *Ulva mutabilis* and German *Ulva compressa* resulted in congruent results proving that a certain morphogenetic mutation pattern is shared. Spontaneous mutations of “slender-like” thalli are appearing whilst the common form exhibits a “leaf-like” wildtype morphology. (2) Interbreeding experiments of gametes of *Ulva compressa* and *Ulva mutabilis* were successful and showed a fertile first-generation offspring exhibiting the typical wildtype morphology similar to the phenotype of the parental generation. (3) Phylogenetic and species delimitation analyses were carried out on 128 *tufA* sequences of *Ulva compressa* specimens sampled in 2014–2016 in Germany and on *tufA* sequences of two clones of the strains *Ulva mutabilis* (sl-G[mt+]) and *Ulva mutabilis* (wt-G[mt-]) to identify Molecular Operational Taxonomic Units (MOTUs). The Generalized Mixed Yule-Coalescent (GMYC) method comprises one major MOTU containing all included sequences of *Ulva compressa* and *Ulva mutabilis*,

while reference sequences included in the analysis clustered outside this MOTU. This highly supports the monophyly of *Ulva compressa* and *Ulva mutabilis*, which can be treated as the same species. As a consequence, *U. mutabilis* is also a suitable model for future studies of green tides and their molecular and morphogenetic basis in the Baltic Sea.

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PROGRESS OF THE SEAWEED MARICULTURE INDUSTRY IN ALASKA

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The mariculture of seaweeds has only recently been initiated in the United States. The current popularity of seaweeds as a food source for humans has increased interest in creating commercial seaweed farms in Alaska. Although mariculture operations in Alaska have existed for over 100 years, only recently has there been effort towards the development of the commercial seaweed industry. The success of seaweed mariculture in the state depends on basic and applied research on the target species. Current research is focusing on the mariculture of kelps, specifically *Alaria marginata* and *Saccharina latissima*. Research on *Saccharina* includes both its natural history and applied research for mariculture operations. *Saccharina latissima* growing near Juneau, Alaska behaves as an annual with growth of the juvenile sporophyte beginning in late winter. Beds of *S. latissima* near Juneau have been found mostly in low energy, shallow subtidal areas of mixed mud, shell hash, sand and small rocks. Research on the mariculture of *S. latissima* and *A. marginata* has indicated that outplants on seeded string wrapped around longlines show optimal growth when set out in October/November and harvested in April, corresponding to the start of the spring phytoplankton bloom. Substantial growth occurs throughout the winter and spring with daily growth rates as high as 5%. All outplantings ceased net growth and deteriorated during late spring and early summer. For mariculture it is likely that light is limiting for growth in the winter and nutrients are limiting for growth in the late spring and summer. Last autumn our commercial hatchery produced about 9 km of seed string for three commercial farms. These farms produced about 1,500 kg of commercially sold *Alaria* and 2,900 kg of *Saccharina* using methodologies developed from this research.

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CAN NITROGEN STRESS BE USED TO INDUCE THE ACCUMULATION OF THERAPEUTIC COMPOUNDS IN MICROALGAE?

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Microalgae provide a rich pool of chemicals including fatty acids, carotenoids, vitamins, sterols and antioxidant compounds. Their potential as a source of valuable nutraceutical and pharmaceutical compounds is now recognized. Advances have been made in the large-scale cultivation of microalgae for production of feedstock for biofuel with stress conditions, most commonly nitrogen depletion, to induce lipid synthesis. Can a similar approach of manipulating growth conditions be applied to enhance the yield of high value therapeutic compounds in microalgae? A series of experiments were conducted with *Chlorella* strains grown in a range of nitrogen conditions. The first experiment used a 120 L photoautotrophic and mixotrophic system with biomass harvested on day 7. Growth rate, protein and carbohydrate levels and photosynthetic pigments were highest in *Chlorella minutissima* cultures grown in moderate nitrogen levels (70 mg L⁻¹) while lipid and fatty acid content were higher in cultures grown in low nitrogen (14 mg L⁻¹). Moderate nitrogen levels, especially in the mixotrophic cultures, enhanced the phytochemical content (phenolics, flavonoids, condensed tannins and iridoids measured by colorimetric methods) as well as biological activity (antioxidant, antimicrobial and acetylcholinesterase inhibitory activity) of the extracts. In a second experiment, three *Chlorella* strains were grown in moderate (35 mg L⁻¹) and high (700 mg L⁻¹) nitrogen conditions and harvested on days 5 and 10. Cultures grown in moderate nitrogen accumulated the highest levels of phytochemicals on day 5. These phytochemicals decreased by day 10 with extracts also becoming less potent in the antioxidant, antimicrobial and acetylcholinesterase inhibitory assays. In contrast, the phytochemical content increased from day 5 to day 10 and the extracts became more potent when grown in high

nitrogen conditions, presumably as nitrogen became limiting in the older cultures. These results indicate that moderate nitrogen stress can be successfully applied to induce the accumulation of these therapeutic compounds in *Chlorella*.

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CHEMICAL COMMUNICATION ON A MICROSCALE – DISCOVERING ADDITIONAL ROLES OF N-ACYL HOMOSERINE LACTONES IN DIATOM BACTERIA INTERACTIONS

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Intertidal marine biofilms are dominated by diatoms and bacteria, together forming hot spots for energy conversion in aquatic food webs. In these heterogeneous biofilms diatoms are found in close association with Proteobacteria, a phylum of bacteria that is known to employ N-acyl homoserine lactone (AHL) mediated Quorum Sensing (QS). Within the biofilm matrix, AHLs have the potential to accumulate to high local concentrations with a fraction of AHLs being degraded into tetramic acids (TA). However, as yet little is known about the effect of these two compound classes (i.e. AHLs and TAs) on the associated diatom community. Here we present results showing that AHLs can have a growth inhibitory effect on diatoms, suggesting that bacteria can control diatom growth in marine benthic biofilms. A screening of synthetic AHLs on the diatoms *Phaeodactylum tricornerutum*, *Seminavis robusta* and *Cylindrotheca closterium* revealed that the observed growth effects were linked to the length of the acyl side chain and the functional group of the molecule. To investigate the effect of tetramic acids on diatoms, here we tested N-(3-oxododecanoyl)-L-homoserine lactone (OXO12) and its derived tetramic acid, 3-(1-hydroxydecylidene)-5-(2-hydroxyethyl)pyrrolidine-2,4-dione (TA12) on *P. tricornerutum*. While diatom growth and photosynthetic efficiency was little affected by OXO12, TA12 addition led to strong growth inhibition, cell death and an immediate drop in photosynthetic efficiency. As a next step, using AHL reporter strains,

we screened 80 bacterial isolates from diatom cultures and marine sediments, of which we identified ten as being AHL producers. These isolates are currently being tested in co-culture experiments with *P. tricornerutum*, *C. closterium* and *S. robusta*, with the aim to set up a model system consisting of a diatom and an AHL producing bacterium. Ultimately, we want to elucidate if and how diatoms and bacteria can engage in AHL mediated crosstalk.

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MULTI-DECADAL TRENDS IN BALTIC SEA PHYTOPLANKTON COMMUNITIES

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The joint Baltic Marine Monitoring Programme started in 1979 by Helsinki Commission has been collecting data on the Baltic Sea phytoplankton for nearly 40 years. During this time, the Baltic ecosystem has undergone substantial changes. Analysis of phytoplankton monitoring data is a valuable tool for understanding both natural and human-induced changes, as phytoplankton plays a key role in biogeochemical cycling and the transfer of energy through the food web. Multi-decadal trends of and relationships between the biomass and community composition of phytoplankton, other biota (zooplankton, benthos, fish) and environmental factors (hydrography, nutrients) were statistically analyzed in the northern, southern and central Baltic Sea. The main goal was to detect changes in the plankton community and/or food web structure and examine the causative factors. Phytoplankton showed significant trends for both total and taxon-specific biomasses. The trends differed between taxonomic groups and Baltic sub-basins, e.g. late-summer cyanobacterial biomass showed an increase in the north but not in the south. In the north, biomass increases of chryso-, chloro- and prymnesiophytes were found, whereas eugleno- and cryptophytes decreased. In the south, there were alternating oscillations between spring biomasses of diatoms and dinoflagellates. Although monitoring data does not allow for inferring straightforward cause-and-effect, multivariate statistics indicated that surface water warming was the most important single factor with respect to changed phytoplankton community structure. Other factors included surface water desalination (and increased stratification), eutrophication (including changed nutrient ratios), and community or biomass changes in other biota. Recently, the potential use of phytoplankton community composition in marine food

web assessments due to the EU Marine Strategy Framework Directive was explored. In the proposed approach, existing knowledge on links between functional characteristics of phytoplankton and food web functioning were combined with methods from long-term data analysis.

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SEXUAL REPRODUCTIVE STRUCTURES OF LOBOPHORA SP. (DICTYOTALES, PHAEOPHYCEAE) FROM CHINA

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In recent years, molecular phylogeny has revealed very rich species diversity in the brown algal genus *Lobophora*. More than 20 new species have been described and the occurrence of about 100 species is estimated based on molecular phylogeny. However, sexual reproductive structures of the genus have been poorly understood. In the present study, we report the male and female reproductive structures of *Lobophora* for the first time based on the newly collected gametophytes in April 2017 from Sanya, Hainan Island, South China Sea. The thalli grew prostrately on rocks at 5 m. Gametophytes are dioecious, with gametangial sori scattered on the dorsal surface, similar to sporangial sori. Oogonial sori are projected above the dorsal cortex without paraphyses, smaller in size and more densely distributed than sporangial sori. Clavate oogonia arise from the cortical cells, 30–50 µm long and 8–12 µm in diameter. Antheridial

sori are small blister-like patches and not bordered by sterile cells. Antheridia also arise from each cortical cell and are of similar diameter, 75–90 µm long and 20–30 µm in diameter. Antheridia are composed of more than 10 tiers of locules. Each locule contains one spermatozoid. Sexual reproductive structures of *Lobophora* are morphologically similar to those of *Zonaria*, but distinct in lacking paraphyses in gametangial sori (excl. *Z. angustrata*).

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NEW GENUS AND SPECIES BASED ON THE CHONDRIA TENUISSIMA AUCT. JAPAN (RHODOMELACEAE, RHODOPHYTA)

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Chondria tenuissima (Withering) C. Agardh (1817), currently regarded as a taxonomic synonym of *Chondria capillaris* (Hudson) M.J. Wynne, is the type species of the genus *Chondria*. This species is characterized by its terete thalli, fusiform branchlets and acute apices and is widely distributed while the type locality is in Portland, England. In Japan, *C. tenuissima* was firstly reported by Okamura (1936). But, descriptions of Japanese *C. tenuissima* include some differences from those of the typical *C. tenuissima* in the literature, which led us to question the identity of this species in the country. We re-examined the Japanese *C. tenuissima* to clarify its classification and phylogenetic position. Morphological results demonstrated that the Japanese *C. tenuissima* differs from the typical *C. tenuissima* in having stoloniferous branches, adventitious pericentral cells among major 5 pericentral cells and no marked spur at the base of a mature cystocarp. Phylogenetic analysis based on *rbcL*, SSU *rRNA* and *cox1* gene sequences showed corresponding results, that the Japanese *C. tenuissima* differs from the typical *C. tenuissima* (as *C. capillaris*) and is separated from the *Chondria* complex. Hence, we conclude that the Japanese *C. tenuissima* is misidentified as *C. tenuissima* and represents a novel taxon. Further, our molecular results suggest that the Japanese *C. tenuissima* should belong to a distinct genus other than the genus *Chondria* in the Rhodomelaceae.

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A SILICOFLAGELLATE VARIABILITY PLEXUS IN THE LATE MIOCENE, EASTERN EQUATORIAL PACIFIC

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Silicoflagellate skeletons are known for a morphological variability, sometimes focused in short time intervals termed plexus events. An unusual variability plexus occurs over approximately 10,000 years in late Miocene sediments (~6 million years ago) of the Eastern Equatorial Pacific. This variability event occurs within morphogenus *Bachmannocena*, which usually lacks any apical structures whatsoever. Several skeletal morphologies (*Bachmannocena diodon nodosa* varieties *cocoensis*, *neonautica* and *transcenna*) that have an arch at various locations between two minor-axis basal sides are part of the plexus. We have examined the stratigraphic distribution and changes in abundance of these skeletal morphologies at high stratigraphic resolution. Six variant morphologies have been counted and show a consistent temporal pattern, with elongate basal rings and arches across the minor axis at the bottom and less elongated rings and the arch moving towards a basal corner higher in the sequence. This study offers a perspective on silicoflagellate variability as sometimes observed in the open ocean and fossil record. Silicoflagellate plexid events are usually interpreted to result from some kind of environmental perturbation. The paleoenvironmental significance of the late Miocene equatorial Pacific plexus, however, remains to be explored.

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SEAWEEDS PROTEIN QUALITY COULD BE BETTER AND THE SHADOW PRICE OF PRODUCTION LOWER THAN IN TERRESTRIAL PLANTS

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Demand for food and feed protein would require raising annual production by 70%, to 1.6 billion metric tons by 2050. The sources of this protein will be determined by cost, market and shadow prices (the monetized external costs, e.g., energy, water, social and environmental implications, being imposed on society as a function of protein production), and the nutritional quality. Seaweed protein content in the biomass can approach 50% (in DW), which

is between cereals and soy. Protein quality in seaweeds, expressed by its chemical score, compares well with the main terrestrial food plants. While animal proteins have a chemical score of 1.0, *Ulva intestinalis* protein has a score of 0.91, higher than corn and wheat (0.47), soy (0.79) and rice (0.86). Red seaweeds protein score ranges from 0.75 to 0.87.

Digestibility (Pronase method) of seaweed protein ranges from 78% (*Porphyra tenera*) to 87% (*Undaria pinnatifida*) and 95% (*Ulva pertusa*). In comparison, digestibility in the common land plants ranges from 70% to 100%.

Market price of seaweed protein is higher than land plants protein (€ 10–20 kg⁻¹ for specific functional proteins) but this is likely to change, following emerging global shortages in agriculture and fisheries production, and rising awareness of the shadow price. Seaweed production inputs and shadow price are both lower than in land plants. For instance, chemicals (fertilizers and pesticides) account for a third of the production cost in US agriculture, but would be much lower in seaweed production. Grain production uses 1–2 m³ water kg⁻¹, compared with none in seaweed production. Corn and soy production inputs impose on the environment additional cost (shadow price) of 25–33%, much more than would be in seaweed production. Therefore, both the protein quality and the awareness of shadow price involved in production will increase attractiveness of seaweed protein for food and feed.

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JAPAN'S NATIONWIDE LONG-TERM MONITORING SURVEY OF SEAWEED COMMUNITIES: TEN-YEAR OVERVIEW AND FUTURE PERSPECTIVES

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“Monitoring Sites 1,000” – Japan’s long-term monitoring survey was established in 2003, based on the proposal outlined in the “Japanese Government National Strategy for the Conservation and Sustainable Use of Biodiversity”. Ecological surveys have been conducted on various types of ecosystems at approximately 1,000 sites in Japan for 15 years now, and are expected to be carried out for 100 years. Since 2008, seaweed communities have been monitored at six sites, featuring the typical *Sargassum* and kelp (*Saccharina*, *Eisenia* and *Ecklonia*) forests in the subarctic and temperate regions of Hokkaido, Honshu and Kyushu Islands. Survey at each site is carried out annually during the season when seaweeds are most abundant. It is a non-destructive survey recording the species diversity and percent coverage in each permanent quadrat, combined with a line-transect survey to reveal the community structure and species composition including vertical distribution of seaweeds. A more intensive survey including destructive samplings is conducted every 5 years. A shift in the community structure as well as in the gap dynamics, as influenced by environmental changes had been observed at some sites. For instance, pre- and post-disaster data revealed the effect of the 2011 Japan earthquake and tsunami. Ground settlement brought about by the earthquake resulted in a shift of the vertical distribution of *Eisenia bicyclis* to shallower depths at the Shizugawa (Minami Sanriku) site facing the Pacific coast in the northern Honshu Island. For almost 10 years, more than 40 specialists in phycology and underwater ecosystems have contributed to the annual survey at six sites. Shortage of contributors to the survey is a serious concern, especially for future generations, suggesting that education and understanding of the biodiversity of coastal ecosystem are essential.

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THE EFFECT OF PAR AND TEMPERATURE ON THE PHOTOSYNTHESIS OF A TEMPERATE JAPANESE BROWN ALGA, *SARGASSUM PATENS*, BASED ON FIELD AND LABORATORY MEASUREMENTS

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The effects of photosynthetically active radiation (PAR) and temperature on the photosynthesis of a temperate Japanese brown alga, *Sargassum patens* (Fucales) were determined by field and laboratory measurements. Dissolved oxygen sensors and pulse-amplitude modulated (PAM) fluorometry were used for the measurements of photosynthetic efficiency. A Diving-PAM revealed that underwater measurements of the effective quantum yield (Φ_{PSII}) for the natural community in Kagoshima, Japan declined with increasing incident PAR, with minimum Φ_{PSII} occurring during noon to early afternoon. Φ_{PSII} recovered in the evening, indicating photo-adaptation to excessive PAR. In laboratory experiments, Φ_{PSII} also decreased under continuous exposure to 200 and 1,000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ at 8, 20 and 28°C. However, full recovery occurred after 12 hours of dark acclimatization only for the treatments 20 and 28°C, suggesting that the combined effect of PAR and cold temperature might have influenced the recovery of Φ_{PSII} . The net photosynthesis–PAR experiments conducted at 8, 20 and 28°C revealed characteristic *P-E* curves, with lower P_{max} value at 8°C. Gross photosynthesis (*GP*) and dark respiration experiments determined over a range of temperatures (8–40°C) revealed the maximum *GP* rate occurring at around 20°C. Maximum quantum yield (F_v/F_m) of *S. patens* was stable at 8–32°C, and decreased thereafter, up to 40°C. This species can be found in the temperate region of Japan with the temperature range between 8–28°C. While this species is probably well-adapted to the current range of seawater temperature, they are also near the boundary of their tolerable limits.

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THE INTERNATIONAL CODE OF NOMENCLATURE OF PROKARYOTES: 50 YEARS ON AND STILL LEARNING

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The current revision of the International Code of Nomenclature of Prokaryotes (2008) is based on the changes that were initiated at the end of the 1960’s/ beginning of the 1970’s and implemented in the 1975 revision of what was then the International Code of Nomenclature of Bacteria.

Key aspects that have been the subject of discussion among those responsible for other Codes of Nomenclature were setting up what was essentially a set of protected names from the existing literature that could be carried forward from a new starting date (1st January 1980) and the setting up of a centralised system for recording those names that were validly published (available). These basic elements have proven to be useful, but over the following years a number of changes have needed to be made. These have included making sure that authentic biological material is deposited that is intended to represent nomenclatural types at the ranks of species and subspecies, as well as making improvements in the way information is presented when new names are formed, their properties listed and types designated. At the same time it has become apparent that there are a number of errors or misunderstandings that were introduced into the 1975 revision that have persisted to today and particularly affects certain elements that may be described as the logical basis of the Code. While these elements affect the way the Code is interpreted with regards prokaryote nomenclature, there are also misunderstandings outside of these circles that affect the way that the Code is appreciated by those dealing with eukaryote nomenclature.

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BRIDGING THROUGH BIOLOGICAL DIVERSITY, REGULATORY FRAMEWORK AND PRODUCT SPECS FOR ACCELERATED COMMERCIAL LAUNCHES IN HEALTH, NUTRITION AND AGRICULTURE

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Our understanding about the extent of biological diversity is constantly expanding by surveys using molecular markers revealing greater than 8.7 million species (mostly microbial) that coexist and interact to create complex assemblages shaping ecosystems. This functional and genetic diversity creates a vast reservoir for commercial products derived from algae and associated microbiota in multiple industries. To study, collect or profit from these genetic resources there is an international legal framework through the Convention of Biological Diversity and agreements including the Nagoya protocol which establishes the guidelines for benefit sharing. In addition, there are local and regional regulatory and legislative control of biodiversity that require compliance for sample collection, movement across state and international borders, and protection for

intellectual property or risk legal consequences and damage the business and brand. The development of products such as DHA from algae, fluorescent proteins from marine invertebrates for diagnostics or biofertilizers for row crops from endophytes has been enabled by a clear definition of product specs and the survey of natural habitats where these environmental conditions promote such adaptations. Recognizing strong biological signals in nature and establishing stringent assays to select for strong lead candidates reduces the screening space and shortens the discovery stage. An understanding of the incumbent technology, benchmarks and products to be replaced by the inventions is essential to access the addressable markets or to create a new market. Beyond the technical aspects of production, scale up and manufacturing it is necessary to have access to distribution channels to provide a solution delighting costumers and growing the demand for the product and upgrades. The new industry created by the microbiome sciences motivated by the health benefits conferred and regulated by microorganisms present in digestive tracts of humans and animals will expand and accelerate discoveries derived from algae. These approaches to commercialize microbial-derived products enabled launches in relatively rapid cycles that can be replicated and adapted to the current markets.

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FRIENDS NOT FOES: UNRAVELING THE ECOLOGICAL RELATIONSHIPS BETWEEN MICRO AND MACROALGAE IN LOW ORDER TROPICAL STREAMS

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Stream beds are richly colonized by benthic algal assemblages. To date, there is a wealth of information suggesting that negative interactions, such as competition, are an important determinant of species composition in stream algae communities. However, considering micro- and macroalgae, our findings indicate the opposite is true during a colonization process. Here, we show that micro- and macroalgae are actually members of the same algal community and they only represent different successional stages, microalgae being early-successional settlers and macroalgae the late successors. This new perspective changes some ecological concepts for stream macroalgae, especially those from tropical regions. For example, for at least two decades, many studies have explained that the autumn/winter seasons are

the best times to find higher richness and abundance of lotic macroalgae, and the reasons is the variation pattern of some climatic and environmental factors. We suggest that there are more macroalgae during the autumn/winter seasons because there are more areas of stream bed reaching late-successional stages owing to more enduring/stable conditions.

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EVOLUTION AND CHARACTERIZATION OF MANNITOL BIOSYNTHETIC PATHWAYS IN ALGAE

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Mannitol, probably the most abundant polyol occurring in nature, is produced by a wide range of living organisms with the exception of Archaea and animals. It fulfils key physiological roles including carbon storage and protection against environmental stress. Among Eukaryotes, plants producing mannitol rely on mannose-6-phosphate (M6P) isomerase, M6P reductase and mannitol-1-phosphatase (M1Pase, uncharacterized yet) for synthesis from fructose-6-phosphate (F6P). Apicomplexa and algae have evolved a different pathway where mannitol-1-phosphate dehydrogenase (M1PDH) reduces F6P into mannitol-1-phosphate (M1P), which is hydrolysed to mannitol by mannitol-1-phosphatase (M1Pase). A similar pathway occurs in fungi, where M1Pase has not yet been identified. Phylogenetic analysis indicated that bacterial/fungal and apicomplexa/algae M1PDHs form two distinct groups among the polyol-specific long chain dehydrogenase/reductase family. Moreover, algal and apicomplexa M1Pases belong to distinct families of phosphatases: the haloacid dehalogenases (HAD-M1Pase) and the histidine phosphatases (His- M1Pase) respectively.

Mining of transcriptomic and genomic resources using sequences of biochemically characterized algal and apicomplexa M1PDHs and M1Pases revealed an unexpected prevalence of mannitol biosynthetic genes across algal lineages and an ancient origin, possibly, in some cases, back to their non-photosynthetic ancestors. It also highlights examples

of genes acquired and adapted through lateral gene transfer between algal groups. In addition, several pathways can co-exist in some algae, including M1PDH/M1Pase fusion proteins, and some species contain several genes coding for the same enzymatic activity. As an example, M1PDH/HAD-M1Pase fusion and standalone HAD-M1Pase co-occur in the green alga *Micromonas*. Heterologous expression showed that both enzymes catalysed mannitol production, supporting results of the census analysis and paving the way for further biochemical characterization. Apparent functional redundancy may be of physiological importance, and raises intriguing questions about evolution, subcellular localization, regulation, and ecological significance of algal mannitol synthesis pathways in the context of carbon assimilation and climate change.

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ASSESSMENT OF QUALITY OF THREE MARINE BENTHIC HABITAT TYPES IN NORTHERN BALTIC SEA

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The European Union Habitats Directive (HD) obliges member states to assess the conservation status of marine habitat types, but no explicit methodologies for assessing the quality of habitats have been stated in the directive or accompanying documents. In this study, a system was developed to assess the structure and functioning of three important marine habitat types in the Estonian sea area: sandbanks (HD code 1110), mudflats (1140), and reefs (1170). The assessment system includes a list of ecological criteria and favourable reference values together with procedural rules. The habitat types listed in the HD are broadly defined and may encompass different communities depending upon distinct environmental gradients. By considering these environmental gradients the habitat types reefs and sandbanks were zoned and the assessment criteria and corresponding favourable reference values were defined separately for each zone. A set of several metrics like benthos indices, community variables, presence of sensitive or typical species, proportions of functional or taxonomic groups were tested as potential criteria for determining habitat quality. The most appropriate criteria were selected for incorporation into the assessment system based on ecological eligibility, suitability to local conditions, occurrence

rates of benthic species, responses to disturbances, statistical properties of distribution of measured values, and practical considerations. An extensive benthos database (records from the whole Estonian sea area, 1995–2014) was used to calculate values of criteria to support the selection of criteria and to derive reference values. In order to fully take into account their crucial role in maintaining the structure and functioning of habitats, the criteria on habitat-forming species were assigned higher hierarchical order in the assessment scheme compared to other criteria. The main challenges of the study were to derive assessment criteria and favourable reference values that are ecologically relevant and practically feasible.

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DNA BARCODING OF LITHOPHYLLOIDEAE (CORALLINALES) REVEALS UNKNOWN DIVERSITY IN BRAZIL

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Multiple-marker (COI-5P, UPA, psbA and rbcL-3P) and variable algorithmic approaches (Automatic Barcode Gap Discovery, Poisson Tree Process and divergence rates) were used for species delimitation of Lithophylloideae. The integrative approach was mostly congruent between markers and algorithmic methods of species delimitation, suggesting the occurrence of 24 species. Based on morphology and molecular data, *Amphiroa rigida*, *A. vanbosseae*, *Lithophyllum atlanticum*, *L. corallinae*, *L. margaritae*, *Titanoderma pustulatum*, *T. prototypum* and *Paulsilvella huveorum* were previously reported for Brazil and are confirmed in this work. Five new species are distinct by both molecular and morphological traits, and they are provisionally named as *Amphiroa* sp. 1, *Amphiroa* sp. 2, *Amphiroa* sp. 3, *Lithophyllum* sp. 1 and *Lithophyllum* sp. 2. Another ten species are cryptic and cannot be distinguished based on traditionally used morphological traits. These include *Lithophyllum* sp. 3, three species that are morphologically named under *A. beauvoisii*, and six that share the morphology described for *A. fragilissima*. The four markers used were useful for species delimitation. However, a combination of practical aspects and levels of intra- and interspecific divergence

values led to the acceptance of rbcL-3P as the preferred DNA barcode for Lithophylloideae.

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A FOUR-GENE APPROACH TO THE BACILLARIACEAE: ESTABLISHING A FRAMEWORK FOR CLASSIFYING A HIGHLY DIVERSE AND TAXONOMICALLY DIFFICULT DIATOM GROUP

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Currently, *Nitzschia* Hassall is probably the largest genus of diatoms, with around 1000 species. In 1880 Grunow published a monograph of the then known (c. 140) species, splitting the genus into 24 sections, but although several changes have been made to its classification since then (e.g. erection of genera such as *Pseudo-nitzschia*, *Fragilar-iopsis*, *Psammodyctyon*), there has been no further comprehensive account. *Nitzschia* and related genera (comprising the Bacillariaceae) are highly diverse with respect to size (2–600 µm), ecology (including freshwater, brackish and marine species, occurring in both planktonic and benthic habitats; some species recognised as bioindicators of organic or metal pollution), physiology (phototrophs, heterotrophs, mixotrophs), and life cycle (e.g. homo- and heterothallic, automictic, avoiding size reduction). They are also notorious for taxonomic difficulty, many having delicate structure and few diagnostic characters in LM or SEM. We have assembled a four-gene data set (nuclear LSU and SSU, plastid *rbcL* and *psbC*) of Bacillariaceae, analysing it gene-by-gene and as a concatenated dataset. Some well supported clades emerge. A few of Grunow's groups agree well with these clades (e.g. his 'Panduriformes'), one of them better than the groupings suggested by later authors (e.g. his separation of 'Apiculatae' and 'Tryblionella', better than *Tryblionella* sensu Mann). Others agree poorly with the molecular trees, especially the very species-rich but character-poor section Lanceolatae. We can identify some

morphological characters, previously little used or ignored, that seem to correlate well with molecular groupings. However, several major divergences in the tree seem to have no morphological signature, so that it will be a major challenge or impossible to develop a classification that both reflects evolution and at same time comprises genera and sections that can be easily recognized in the microscope.

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RESISTANCE TO FROST OF CONJUGATING GREEN ALGA ZYGNEMA (ZYGNEMATOPHYCEAE) TESTED BY AN EXPERIMENTAL APPROACH

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Zygnematophycean green algae have a wide distribution and can form a dominant part of many hydro-terrestrial ecosystems, where they frequently encounter environmental stresses such as high irradiation, desiccation or low temperatures. Effects of desiccation and UV irradiation have been studied in strains from the High Arctic. However, knowledge about their ability to cope with freezing temperatures is still missing. *Zygnema* sp. can produce high amounts of biomass and form characteristic, extensive mats. It has been confirmed by many field observations that mats occur only seasonally and have a temporary character. We tested frost resistance of *Zygnema* sp. strains of different culture age (2 weeks and 8 months) using programmable freezers that enabled us to set various parameters – cooling rate, warming rate, target temperature and time of exposure. We exposed young cultures to freezing temperatures down to -14°C , whereas old cultures were frozen down to -70°C . Survival rate of cells after freezing was tested using fluorescence staining with 0.1% Auramine O dye and measurement of photosynthetic activity by steady-state quantum yield of PSII in the light (ΦPSII). Young cultures showed recovery after freezing from -6°C to -8°C , whereas old cultures could even survive freezing at -30°C to -40°C . Cryo-microscopical observations and ultrastructure examination allow us to visualize the cellular effects of freezing temperatures and frost injury. Our results show that frost hardening via formation of pre-akinetes is crucial

for survival in the harsh temperature conditions prevailing in the High Arctic and plays a key role in the seasonal dynamics of this alga.

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DIVERSITY OF THE MICRASTERIAS PAPILLIFERA/RADIOSA (DESMIDIALES) SPECIES COMPLEX

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We investigated diversity within the *Micrasterias papillifera* *M. radiososa* (Desmidiaceae) species complex. Both species are inhabitants of endangered biotopes, such as peat-bogs and, like other desmids, are used for biomonitoring. Many described varieties for both species can be found in taxonomic literature, but their traditional morphological descriptions are often overlapping and revision of this species complex seemed to be necessary. All strains were isolated from peat-bogs and lakes across Europe and the dataset was completed with strains from algal collections. Results of phylogenetic analysis of sequences for ITS and *psaA* revealed that the real intraspecific diversity of *Micrasterias papillifera* Brébisson ex Ralfs was overestimated. Although we could find a little intraspecific diversity within the ‘*papillifera*’ lineage in the phylogenetic tree based on ITS sequences, it did not correspond with the morphology of isolates. *M. papillifera* possesses unexpected morphological plasticity and also some isolates designated as *Micrasterias radiososa* Ralfs were nested in this lineage. In contrast, there were only a few isolates in the lineage ‘*radiosa*’. This species seems to be relatively rare and probably has limited distribution in North Europe and the British Isles. Both species are morphologically well delimited from each other on the basis of several characters (cell size and position of spines on the cell wall) and can be relatively simply determined.

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ALGAL MICROFOSSIL ASSEMBLAGES FOUND IN HOLOTHURIAN GASTROINTESTINAL TRACTS

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Holothurians (sea cucumbers) are indiscriminate detritus feeders, seemingly ingesting anything on the seafloor, and are thus important members of the megabenthos. In this study the contents of the anterior and posterior parts of the guts were investigated in five species of holothurians: *Holothuria forskali* from Peyrefite Bay, western Mediterranean, *H. atra* from Okinawa Main Island, *H. leucospilota* from Taketomi-jima and Nagasaki, *H. japonica* from the East China Sea, and *Bohadschia marmorata* from Ha'a o Tupa Bay, Nuku Hiva Island. In all cases, there was a clear difference between the anterior and posterior gut contents: the anterior part contained a mixed microfossil assemblage, while the posterior part retained only the siliceous components (diatoms, silicoflagellates etc). This suggests that the calcareous, chitinous and organic components were digested (dissolved) by acidic conditions in the foregut, while the siliceous component remained intact. In most cases the calcareous nannofossils were considerably older than the diatoms, suggesting that the former were transported by rivers, whereas the latter were deposited in situ in coastal waters. The diatoms included modern marine benthic (e.g., *Mastogloia*, *Diploneis*, *Navicula*, *Surirella*) and planktonic (e.g., *Actinocyclus*, *Thalassiosira*, *Bacteriastrum*) taxa. The nannofossils in the holothurian from Peyrefite Bay ranged in age from Jurassic to Holocene, with the Jurassic nannofossils most likely transported from the upper catchment of the Rhône River (since most of the other rivers and Rhône tributaries cut through younger outcrop or non-fossiliferous sediments). The *H. forskali* intestines also included *Corbisema* sp., an extinct species from the Miocene or early Pliocene.

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CPMINUS1, MT- GENOME SPECIFIC GENE OF HETEROThALLIC CLOSTERIUM PERACEROSUM-STRIGOSUM-LITTORALE COMPLEX, IS INVOLVED IN SEX DETERMINATION

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Sexual reproduction is a process that mixes genetic information and produces new combinations, which could thereby accelerate adaptive evolution. Although many dioecious species have sex chromosomes or sex determining regions, the mechanism of sex determination has been reported only in persimmons. We focused on a unicellular charophycean alga *Closterium peracerosum-strigosum-littorale* complex (*C. psl.* complex). The heterothallic *C. psl.* complex has two morphologically indistinguishable sexes: mating type plus (mt+) and mating type minus (mt-). Their sexes are genetically determined. Sexual reproduction is induced when cells of the two sexes are cultured together in nitrogen-depleted medium. During the processes, they recognize each other, become paired, release their protoplasts and finally form a zygote. From the transcriptomic and genomic data, we found a mt-genome-specific gene encoding a transcription factor and named *CpMinus1*.

To evaluate the function of *CpMinus1*, a cDNA with a complete coding sequence was cloned and inserted into the expression vector (pSA0104) for the *C. psl.* complex. The linearized construct for the ectopic expression was introduced into the mt+ strain. Among the six stable transformants, four showed a sexual reaction without mating partners, as observed in homothallic strains. The other two formed zygotes with mt+ strain but not with mt- strain. The gene expression profile of transformants, as determined by quantitative PCR and comparative transcriptome, showed the characteristic pattern seen in the wild-type mt- strains. In addition, we applied CRISPR/Cas9 system to obtain *CpMinus1*-knockout mutants in mt- background. Among six transformants, five had frame-shift mutations and tended to form zygotes with mt- but not with the mt+ strain. The gene expression profile of these knockout mutants was similar to that of wild-type mt+ cells. From these results, we concluded that the *CpMinus1* gene determined the sex of *C. psl.* complex and was responsible for both the expression of the mt- phenotype and the suppression of the mt+ phenotype.

SPATIO-TEMPORAL VARIATIONS IN PHYTOPLANKTON COMPOSITION OF LAKE BAFA, TURKEY

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Lake Bafa is the largest lake in western Anatolia. Despite Lake Bafa being a protected area it is affected by environmental pollutants, especially from river flows. The water quality of the lake depends on Menderes River. This river is polluted by industrial, domestic and agricultural pollutants. The negative changes in water quality severely affect hydrophytes, aquatic birds, fishes, pelagic organisms and benthic organisms along with users of Lake Bafa as a recreation area and local people living around the lake.

Water sampling was implemented in the second week of the month for a year. Eight different stations were determined for sampling. Sampling was conducted horizontally with a 50 µm mesh-size plankton net and samples were stored in Falcon tubes with formaldehyde solution. Quantification and species identification of phytoplankton samples were done under a light microscope. *Chaetoceros muelleri*, *Nitzschia acicularis*, *Ulnaria ulna* and *Coscinodiscus granii* were determined as the main phytoplankton species of the Lake Bafa.

Multi Dimensional Scaling (MDS) showed that the four stations which are distant from the estuary and have greater depth formed a group, while the others showed a separate grouping regarding species composition and abundance. When considering season we obtained three different species groupings: summer, autumn – early winter and spring – late winter. May and September stood out as transitional months. Even though December and January are concurrent months, they had the lowest similarity in species composition, which highlights that each year may be represented by varying species composition. Preliminary results indicate that distance from the river outflow, depth of water column and season are the factors to use as indicators of environmental impacts that effect the phytoplankton species composition of Lake Bafa.

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CHANGES TO THE INTERNATIONAL CODE OF NOMENCLATURE FOR ALGAE, FUNGI, AND PLANTS MADE AT IBC2017 IN SHENZHEN: WHAT DOES THIS MEAN FOR YOU?

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The rule book for naming organisms in phycology, mycology, and botany is the International Code of Nomenclature for algae, fungi, and plants, which is amended every six years at the 5-day Nomenclature Section of an International Botanical Congress (IBC). The current edition of these rules, the Melbourne Code (<http://www.iapt-taxon.org/nomen/main.php>), resulted from the XVIII IBC held in Melbourne, Australia in 2011. The Code will be amended next at the XIX IBC (IBC2017), to be held in Shenzhen, China in July 2017. Even though the new Shenzhen Code will not be published until 2018, its rules will take immediate effect – except where explicitly limited by date – on the last day of the IBC, when a plenary session votes on a resolution to approve the decisions and appointments of the Nomenclature Section. One particularly important issue to be discussed and voted on is the mandatory registration of names of algae and plants, a requirement that has applied to the naming of fungi since 2013. This presentation will be one the first reports on the major changes to the Code resulting from IBC2017, with particular emphasis on the nomenclature of algae.

CELL STRUCTURE AND ULTRASTRUCTURE ADAPTATION OF NOSTOC COMMUNE VAUCHER EX BORNET & FLAHAULT AS A RESPONSE TO DIFFERENCE LIGHT INTENSITY

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Nostoc commune is a terrestrial edible blue-green alga (cyanobacteria) that has a cosmopolitan distribution. In Java, *N. commune* is endemic in the limestone area and forms green-brownish jelly-like clumps or wads on soil, during the rainy season. We found that the *N. commune* living in low light intensity and high light intensity formed two distinctly morphological colony appearances because of an adaptation of being exposed to differences light intensity. However, apart from distinct morphological appearances of the colony, the morphological structure of vegetative cell, akinet and heterocyst do not show any morphological changes. The ultrastructural characterization of both distinct morphological colony appearances showed that the vegetative cell of *N. commune* was covered by fibrillar and granular mucilage on the outside of the cell wall. The cell wall consisted of four layers. The inner side of cell membrane consisted of chromatoplasm and nucleoplasm. The chromatoplasm (cytoplasm) consisted of thylakoid lamellae, cyanophycin and polyglucan granules, polyphosphate bodies, lipid droplet and gas vacuoles. Moreover, the nucleoplasm (nuclear region) consisted of a matrix of fibrillar DNA, ribosomes, and polyhedral bodies. The heterocyst had a thylakoid which spread through the whole cell protoplasm and formed a reticulate structure, without any polyphosphate body, polyhedral body, or cyanophycin granules. The akinet cell had a contorted thylakoid that spread through the whole protoplasm, accumulated a number of cyanophycin granules, and was without any lipid droplets or polyhedral body. The *N. commune* that had been exposed to high light intensity showed a thylakoid concentrated in the periphery of the cytoplasm, thicker fibrillar matrices, and more gas vacuoles than the one that exposed to low light intensity. A detailed description of the cell structure and ultrastructure adaptation characters of *N. commune* in response to difference in light intensity is presented in the article. Key word: *Nostoc commune*, cell structure, ultrastructure

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HYBRIDIZATION IN NATURAL POPULATIONS OF TWO SEaweEDS OF THE GENUS *ECTOCARPUS*: ANALYSING PRE- AND POST-ZYGOTIC BARRIERS IN HAPLOID-DIPLOIDS

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We aimed to study the importance of hybridization between two cryptic species of the genus *Ectocarpus*, a group of filamentous algae with haploid-diploid life cycles that includes the principal genetic model organism for the brown algae. In haploid-diploid species, the genetic structure of the two phases of the life cycle can be analyzed separately in natural populations. Such life cycles provide a unique opportunity to estimate the frequency of hybrid genotypes in diploid sporophytes and meiotic recombinant genotypes in haploid gametophytes, allowing the effects of reproductive barriers preventing fertilization or preventing meiosis to be untangled. The level of hybridization between *E. siliculosus* and *E. crouaniorum* was quantified along the European coast. Clonal cultures (568 diploid, 336 haploid) isolated from field samples (10 populations) were genotyped using cytoplasmic and nuclear markers to estimate the frequency of hybrid genotypes in diploids and recombinant haploids. We identified admixed individuals using microsatellite loci, classical assignment methods and a newly developed Bayesian method (XPloidAssignment), which allows the analysis of populations that exhibit variations in ploidy level. Over all populations, the level of hybridization was estimated at 8.7%. Hybrids were exclusively observed in sympatric populations. More than 98% of hybrids were diploid (40% of which showed signs of aneuploidy) with a high frequency of rare alleles. The near absence of haploid recombinant hybrids demonstrates that the reproductive barriers are mostly post-zygotic and suggests that abnormal chromosome segregation during meiosis, following hybridisation of species with different genome sizes, could be a major cause of interspecific incompatibility in this system.

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METABOLOMICS OF BIOTIC INTERACTIONS IN CENTRIC DIATOMS *COSCINODISCUS* SPP.

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Centric and pelagic diatoms *Coscinodiscus* spp. are photosynthetic unicellular eukaryotes characterized by hydrated silicified shells, numerous chloroplasts and disc-shaped cells. Considered as one of the largest marine pelagic diatom genera, *Coscinodiscus* commonly thrive in complex and diverse planktonic communities in oceanic water columns. In particular, bacteria are always found in association with diatoms and act often as potential mutualists, but their impact on diatom *Coscinodiscus* spp. remains totally undiscovered. Furthermore, field studies reported the parasitic oomycete *Lagenisma coscinodisci* as a major pathogen of *Coscinodiscus* spp. occurring during the diatom blooming season. However, while the infection stages have been well described, the molecular interaction and the identity of chemical cues have been previously unexplored and are the subject of this contribution.

In this context, we developed analytical techniques including mass spectrometry and comparative metabolomics in order to investigate chemically mediated interactions between diatoms *Coscinodiscus* spp., the parasitic oomycete *Lagenisma coscinodisci* and cultivable associated bacteria. Thus, we monitored intracellular and extracellular metabolic changes highlighting up- and down-regulated potential biomarkers. The chemical characterization of selected metabolites is pursued with tandem mass spectrometry and with ions matching with a built in-house references database. MS-imaging methods were also developed to monitor single cell responses to biotic interactions, as well as the influence of stress such as silicate depletion. Thorough this interdisciplinary approach, we are deciphering the chemical mediation of planktonic microorganisms at the cellular level by highlighting a host-pathogen relationship and bacteria-diatom mutualistic interaction. The chemical characterization on a molecular level will serve as a basis for future ecological experiments addressing the role of heterogeneity in planktonic communities.

THREE ECOLOGICALLY DIVERSIFIED GREEN ALGAL GENERA ESTABLISHED SYMBIOSIS WITH A SINGLE LICHENIZED FUNGUS

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Symbiosis plays a fundamental role in nature. Lichens are among the well-known symbiotic organisms distributed worldwide whose ecology is shaped by the requirements of all symbionts forming the holobiont. The widespread lichen genus *Stereocaulon* provides a suitable model to study the ecology of microscopic green algal symbionts (i.e., phycobionts). In this study, we aimed to find a connection between the phycobiont diversity in *Stereocaulon* lichens and environmental conditions. Although a single algal genus, *Asterochloris* (Trebouxiophyceae), was associated with the most of sampled thalli, two additional genera were also found within the lichen thalli. First, the common free-living alga *Chloroidium* was recorded as a phycobiont in numerous thalli growing on volcanic or artificial substrates. Second, the recently described genus *Vulcanochloris* was found in several lichens growing on volcanic substrata. We used variation-partitioning analyses to investigate the effects of climate, habitat, and spatial distribution of the three phycobiont genera and their species. According to these analyses, the general climatic conditions drive the distribution of phycobionts at the level of genera or higher. Conversely, the distribution of a particular species is probably determined by microhabitat conditions and substrate characteristics. We show that *Asterochloris* prefers a cold, humid climate, *Vulcanochloris* tolerates warm, extremely dry conditions, and *Chloroidium* tolerates a wider humidity range but prefers moderate-to-warm temperatures. Furthermore, the survival of lichens on substrata with high concentrations of heavy metals appears to be facilitated by the association with toxibiont *Chloroidium* phycobionts. The mycobiont can therefore choose the best adapted alga as phycobiont in each type of habitat.

DMS/P SIGNALING MEDIATES THE CROSS-TALK WITHIN THE COCCOLITHOPHORE PHYCOSPHERE

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Emiliania huxleyi is a globally important coccolithophore that forms massive algal blooms in the North Atlantic Ocean. Despite the huge importance of these phytoplankton blooms to large biogeochemical processes, relatively little is known about the chemical signaling that mediates microbial interactions within the phycosphere. We are interested in understanding the cellular mechanisms that control cell fate during bloom demise, governed by the response of coccolithophores to microbial interactions with viruses, bacteria and grazers.

DMS, a ubiquitous infochemical produced within the coccolithophore phycosphere is a bioactive gas that is released to the atmosphere from algal blooms (~109 tons annually) where it is oxidized to sulfur aerosols that act as cloud condensation nuclei. Although DMS production was known since the 30's, its algal enzymatic source was unknown till now. We recently revealed this critical piece of the puzzle of the oceanic sulfur cycle, by identifying the algal enzyme DMSP lyase in *E. huxleyi*. We are currently investigating the signaling role of DMS/P in mediating interactions of *E. huxleyi* with microzooplankton and during infection by a newly isolated pathogenic bacterium. We propose that regulation of DMSP catabolism during microbial cross-talk may generate specific infochemical compositions with unique biogeochemical imprints.

BIO-PRODUCT AND WASTE WATER/CO₂ REMEDIATION POTENTIAL OF A NITROGEN- FIXING CYANOBACTERIUM

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Carbon dioxide (CO₂) emissions are expected to rise to 40.3 Gt by 2030, with significant impacts on climate. Even though efforts are underway to reduce reliance on

coal-generated energy, most alternative sources are only intermittently available. Thus, fossil fuel-derived energy will still be required in the foreseeable future to ensure stable and demand-scalable energy grids. Flue gas emissions from coal-fired power stations contain ~10% CO₂ and the waste water generated (ash-dam water: ADW) is rich in metals (most being essential trace elements for plants), but otherwise nutrient-poor. All oxygenic photosynthetic organisms possess the ability to sequester CO₂, but only nitrogen-fixing cyanobacteria are suitable for commercial-scale cultivation at coal-fired power stations. Production of non-nitrogen fixing photosynthetic biomass incurs high fertilisation costs, making production uneconomical. Cyanobacterial biomass also represents a promising source for biofuel and bio-product development in a wide range of commercial applications. Here we determined CO₂ and trace metals remediation, and bio-product potential of the nitrogen-fixing filamentous cyanobacterium *Tolypothrix* sp. Biomass production of *Tolypothrix* sp. grown in simulated ash-dam water (SADW) and BG11 medium without nitrogen (BG11(-N) controls) was 2.4 (SADW) and 3.3 (BG11(-N)) times higher when supplemented with 10% CO₂-enriched air, compared with non-CO₂ media controls, yielding 276 and 216 mg dry biomass L⁻¹ day⁻¹. *Tolypothrix* sp. removed ~99% of the 10% CO₂ (flow rate 100 mL min⁻¹) and anti-oxidant pigment content (phycocyanin and phycoerythrin), of interest to the cosmetics industry were 1.3 and 1.2 times higher compared to non-CO₂ controls for the two media, respectively. Biomass yields and productivity, as well as iron accumulation makes *Tolypothrix* sp. a good candidate for CO₂ and ADW remediation at coal-fired power stations; bioproduct potential as biofertiliser, to combat soil infertility of Australian carbon-poor tropical soils; and antioxidant pigment production, as additives for feeds or cosmetics.

THE PHOTOTROPHIC MICROBIOME OF CORAL SKELETONS

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Endolithic microalgae and bacteria living inside coral skeletons play important roles in the calcium carbonate budget and nutrient cycling of coral reefs. We used multi-marker high-throughput environmental sequencing to characterise the diversity of green algae and bacteria in skeletons of

Indo-Pacific corals. Despite being an ultra-low light habitat, we found a highly diverse assemblage of phototrophic microbiota inhabiting coral skeletons, including >100 green and red algal OTUs, cyanobacteria and various other phototrophic bacteria. We discuss the biology and ecology of these organisms as part of the coral holobiont.

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POTENTIAL EFFECTS OF CLIMATE WARMING ON PHOTOSYNTHETIC RESPONSES OF TROPICAL STREAM MACROALGAE

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The increase in global mean temperature due to human influence has become a major concern during the past few decades. Ecological consequences of this anthropogenic climate change are already being reported around the world. Freshwater habitats have characteristics that put their biodiversity at an even greater risk. In low-order tropical streams, benthic organisms contribute a large fraction of the community energy input from primary producers and play an important role in sustaining autotrophic food webs. Using chlorophyll fluorescence and dissolved oxygen evolution techniques, we evaluated the effects of projected temperature increases due to global warming on the photosynthetic response of lotic macroalgae for two future scenarios (RCP 4.5 and RCP 8.5), as proposed by the Intergovernmental Panel on Climate Change (IPCC). Control temperatures were determined in both summer and winter seasons and experimental scenario temperatures were calculated by adding the projected IPCC increase to the seasonal means. In spite of species-specific patterns of responses to the simulated scenarios, some general trends were clearly observed. In general, both chlorophyll fluorescence and dissolved oxygen showed that the IPCC-predicted scenarios (mainly RCP 8.5) would severely jeopardize the photosynthetic performance of most tested Rhodophyta species. In addition, the projected scenarios of the IPCC did not produce significant negative effects on the photosynthetic efficiency of the Chlorophyta species in general, revealing at least a tolerance of green algae to the temperature variations tested. In some cases, for example *Spirogyra*

sp., an increase in the photosynthetic performance of green algae was observed. Given the position of these organisms at the base of the food web, our results indicate that the fulfillment of the IPCC scenarios could cause important impacts to tropical lotic environments as a whole, especially in shaded low-order streams where Rhodophyta constitute a highly relevant group of primary producers.

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SYSTEMATIC REVISION OF THE SISTER GENERA *LEMANEA* AND *PARALEMANEA* (BATRACHOSPERMALES, RHODOPHYTA)

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The freshwater red algal genera *Lemanea* and *Paralemanea* are common members of the freshwater flora in North America and Europe, but have been reported from other continents sporadically. They inhabit streams with high current velocities and grow in lawns on large boulders and bedrock. The two genera are easily distinguishable from each other using multiple morphological characteristics. Although the genera are easily identified, determination of species based on morphology can be challenging. The present study was undertaken to use both molecular tools for species delimitation and to provide morphological characteristics for species identification, whenever possible. For *Lemanea*, 147 sequences were generated with 43 unique sequences used for phylogenetic analyses. Likewise, for *Paralemanea*, 51 and 27 sequences were available, respectively. Phylogenetic analyses showed that each genus formed a highly supported clade. Within genera, there were numerous well-supported clades. Interestingly, most clades contained specimens that were geographically related; in both *Lemanea* and *Paralemanea* the North American specimens were distinguished from the European specimens.

Furthermore, there was a division between eastern and western North America for both genera. The only exception was a specimen from Newfoundland, Canada that was related to two specimens from Finland identified morphologically as *Lemanea condensata*. There were three specimens that were separated from other clades on long branches, including a specimen from the type locality in California of *Paralemanea tulensis*, a yet to be named *Paralemanea* from Kenya and the recently published *Lemanea manipurensis* from India. In previous studies, European names have been applied to North American specimens, but it is clear from our findings that these names are not appropriate. Therefore, North American specimens will be re-evaluated for potential application to new epithets based on the clades. Most of the European specimens will retain the names previously applied in the literature.

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IS IT POSSIBLE TO FUNCTIONALIZE HASLEA OSTREARIA SILICA FRUSTULES WITH POLYISOPRENE CHAINS AND USE THEM AS CHARGES IN THE SYNTHESIS OF NEW ELASTOMERIC MATERIALS?

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The marine diatom *Haslea ostrearia* is currently studied and cultivated for the production of the blue pigment marennine, which is responsible for the greening of oysters in the French region of Marenne Oleron (Fine de claire verte), and which has also been shown to possess antiviral and antibacterial activity. The upscale in the production of the microalgae is currently carried out in view of the multiple applications of marennine and other valuable compounds produced by diatoms. In this framework, the silica frustules will accumulate in bioreactors and be considered as a waste. This work aimed at investigating the possibility of recycling

these frustules' crumbs as inorganic charges to be inserted in the formulation of elastomeric materials to reinforce mechanical properties. The case of natural rubber derived materials has been considered and the frustules have been functionalized with original polyisoprene oligomers to make them more hydrophobic and easier to disperse in the matrix than the unfunctionalized hydrophilic silica particles.

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SELECTION OF INCREASED RESISTANCE AGAINST EPIPHYTISM DURING THE INVASION HISTORY OF GRACILARIA VERMICULOPHYLLA

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Fouling is a stressor that might determine the fate of seaweeds, but reports of algal adaptation to epibiosis are scarce. Previous comparisons have shown resistance to epibionts can be higher in non-native than in resident seaweed species, but we do not know whether it is an intrinsic trait of the non-natives or it has been acquired during the invasion process. We here compared native and non-native populations of the same algal species to elucidate this question. Resistance against two groups of epiphytes was assessed in living thalli and in artificial substrata coated with surface extracts, both gained from four Asian (native) and four European (non-native) populations of the red alga *Gracilaria vermiculophylla*. Two diatom species and two filamentous macroalgae were used as micro- and macro-epiphytes, and one of each type was collected in Asia, while the other came from Europe. Laboratory assays were done in both distributional ranges of *G. vermiculophylla* and in different seasons. We used a fully crossed design with the factors (i) 'Origin of Gracilaria', (ii) 'Origin of epiphytes', (iii) 'Season' and (iv) 'Solvent used for extraction'. Both groups of epiphytes, regardless of their origin, attached less to living thalli and to surface extracts from non-native *G. vermiculophylla*. Fewer diatoms attached to hexane-based extracts, while fewer *Ceramium* filaments settled on extracts gained with dichloromethane. Our results show for the first time that non-native individuals of a seaweed are better defended against epiphytes than native conspecifics. Furthermore, we found evidence that at least a part of the defence is based

on extractable secondary metabolites. We suggest that an enhanced defence against epiphytes after introduction is one reason for *G. vermiculophylla*'s invasion success. Our observation may also apply to other basibiont–epibiont interactions and could be a key feature of seaweed bioinvasions.

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SYSTEMIC ACQUIRED RESISTANCE OF A RED ALGA HETEROSIPHONIA JAPONICA AGAINST OOMYCETE OLPIDIOPSIS HETEROSIPHONIEAE

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A filamentous red alga *Heterosiphonia japonica* shows a systemic acquired resistance (SAR) against an oomycete pathogen, *Olpidiopsis heterosiphoniae*. A cell-type specific infection occurs in *H. japonica*. Host filaments can survive without fatal damage as infection is localized in the distal cells of determinate branch and rhizoid cells. Often host and pathogen can coexist in culture for more than several months. Infected *H. japonica* underwent some morphological and physiological changes. Fluorescent staining showed that infected filament showed reduced growth compared to the control, even when several cells were actually infected with the oomycete. The number of infected cells was decreased to about 40% after the five days of infection. Infected filaments showed much higher tolerance to osmotic stress compared to control. These results suggest oomycete infection in *H. japonica* might help the host to survive in the intertidal environment where osmotic conditions change frequently.

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CALCIUM-DEPENDENT SIGNALLING MECHANISMS IN THE PORPHYRA GENOME

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The rocky shore inter-tidal zone inhabited by *Porphyra* represents a particularly harsh environment, with organisms experiencing extremes of temperature, salinity, desiccation and light on short time scales. The ecological success of *Porphyra* in the inter-tidal zone is therefore dependent on

its ability to sense and rapidly respond to changes in its environment. Ca^{2+} -dependent signalling mechanisms are central in the response of eukaryotes to many stimuli, such as osmotic stress, oxidative stress, light, temperature and pathogens. However, whilst the nature of Ca^{2+} signalling in land plants has been well-characterised, detailed information on the signalling mechanisms present in red algae is currently lacking. The recent sequencing of the *Porphyra* genome has enabled us to analyse the composition of the Ca^{2+} signalling toolkit in this lineage (i.e. the cellular mechanisms that are responsible for the generation and decoding of cytosolic Ca^{2+} elevations). We find that *Porphyra* exhibits some similarities with land plants in classes of Ca^{2+} channels that are present. However, the mechanisms through which *Porphyra* senses cytosolic Ca^{2+} elevations appear to be very different, with no clear homologues of the classes of Ca^{2+} sensor kinases that are present in large gene families in land plant genomes. A wider examination of red algal genomes suggests that red algae may use novel mechanisms to sense cytosolic Ca^{2+} elevations. The unique Ca^{2+} signalling toolkit of the red algae may therefore contribute to distinct signalling pathways in response to environmental stressors in this lineage.

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FROM SEAWEED TO BIOGAS: EXPOSING THE MICROBIOME UNDERPINNING STABLE ANAEROBIC DIGESTION

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Anaerobic digestion (AD) is a core component of progressive strategies for renewable heat and electricity generation, particularly for meeting the demands of rural and isolated communities. AD processes are generally not optimised for unconventional feedstocks, including macroalgae (seaweeds). Furthermore, the high polyphenolics content of phaeophytes (brown seaweeds) are notoriously problematic for the microbe-driven AD process when operated over extended periods (i.e. continuously). It was to this end that we undertook the most comprehensive study to date of continuously operated seaweed anaerobic digesters, operating a series of reactors fed fresh and ensiled seaweed (*Saccharina latissima*), in combination with food waste, for an unparalleled two years. A particular focus was the response

of the AD reactor microbiome to various processing, feeding and operating conditions. Paradoxically, despite AD being a microbial process, a comprehensive analysis of the seaweed AD reactor community underpinned by detailed reactor performance data, is sorely lacking. We have addressed this pressing knowledge gap as part of the ground-breaking SeaGas project.

Digester performance (five litre continuously stirred tank reactors, CSTRs) was unhindered by polyphenol content, yielding a steady biogas supply across all conditions and feedstocks, with yields comparable to established AD processes. Methane output was more stable in reactors fed mixes of macroalgae and food waste, as opposed to those fed macroalgae only. Core microbial assemblages were comparable within all digesters; however, community alpha and beta diversity varied within processing, feeding and operating conditions, highlighting the evolution of the microbial communities through time towards steady state. Using a combination of reactor metadata and functional metabolic pathway analysis, the tight coupling between reactor operation and microbial community stability was elucidated. Scale-up trials (800 L CSTRs) are currently underway to validate reactor-community stability at pilot scale operation. This is the first study to deliver such high resolution time-integrated data.

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THE SEA LETTUCE *ULVA* ONLY GETS INTO SHAPE WITH THE RIGHT BACTERIA: HOW MORPHOGENS DIRECT ALGAL DEVELOPMENT AND DIFFERENTIATION

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Ulva cultures without associated bacteria develop very slowly and do not form the expected morphology known from nature but instead a mass of undifferentiated cells. Two specific types of bacteria, *Roseovarius* sp. and *Maribacter* sp., have been shown to be associated with *Ulva* and influence the development of the alga by releasing morphogenetic compounds (= morphogens). The *Roseovarius* induces the blade cell division so that the typical thallus is formed, thereby acting similarly to the plant hormone cytokine. The *Maribacter* strain triggers the cell wall formation and the differentiation of basal cells into a rhizoid which enables the fixation of the alga to the substratum; thereby this morphogen act similarly to the plant hormone auxin. Besides the bacteria-mediated differences in algal

development, some *Ulva* species often exhibits characteristic morphological changes, which are not related to the associated microbiome. One of this species was named *mutabilis*, because a wide variety of different developmental mutants (called, for example, 'slender', 'long' or 'bubble') spontaneously arose with an unusually high frequency among the offspring of these strains. However, it was realized that only a few of the originally isolated strains possess the trait of the high mutability. I introduce the various aspects of *Ulva*'s plasticity in morphogenesis and show why *Ulva mutabilis* presents excellent opportunities for the detailed study of plant morphogenesis.

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STUDY OF 'PETIT LAC OF ORAN', DAYAT MORSLI AND WATER TREATMENT BY A BIOREMEDIATION APPROACH

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Our study is about a lake 'Petit Lac d'Oran' or 'Dayat Morsli' in Oran (a city located in north-west of Algeria). Dayat Morsli is a small kidney-shaped endorheic salted depression 150 ha in area. This lake is characterized by a wide diversity of plant and animal species, including migratory birds which use it as a resting place. Over time industrial factories and slums have settled all around the site, as a consequence Dayat Morsli is exposed to industrial and urban waste which has led to severe pollution of the site. The municipality of Oran has set up a project to rehabilitate and decontaminate the site by stopping waste and dismantling slums. Our aim is to participate in the bioremediation of the site, which requires identification of the pollutants present in the aquatic environment by measuring five heavy metals; (Cd, Pb, Cu, Zn and Fe), determination of several physicochemical parameters such as pH, salinity, chemical and biological oxygen demand, nitrates and phosphorus and molecular identification of aquatic flora (microalgae, bacteria). The monitoring of physicochemical measurements shows that the lake is polluted with values above standards, especially for nitrates, phosphorus and heavy

metals. Identification with 16S and 18S markers revealed the presence of a rich bacterial flora with several species belonging to different phyla which can be applied in bioremediation, such as *Thiohalomonas denitrificans* and *Flavobacterium nitrogenifigens* which are denitrifying bacteria, as well as cyanobacteria and microalgae specific to a saline environment such as *Dunaliella salina*, *Halamphora coffeiformis*, *Navicula cryptocephala* and *Asteromonas gracilis*.

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FOSSIL RECORD CLUES TO DIATOM RISE TO ECOLOGICAL PROMINENCE – ARE WE LOOKING IN THE RIGHT PLACE?

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In the modern oceans, diatoms are considered the main primary producer group, responsible for up to 40% of net global photosynthesis each year and making a key impact on global carbon and silicon cycling. Most of the modern diatom production, however, is focused on the continental shelves and in major upwelling systems. Despite their present-day importance in global biogeochemical cycles, it remains a matter of speculation when in geological time diatoms rose to ecological prominence. Reconstructing the timing of diatom evolutionary events requires a robust chronology. Long, continuous stratigraphic records with reliable age control are offered by deep-sea sediments. In deep-sea cores, however, Cretaceous and early Paleogene diatoms (~80–40 million years ago) are probably underrepresented due to a number of diagenetic factors acting against opaline silica preservation. As a result, numerous paleoproductivity studies link diatom rise to ecological prominence with the Eocene-Oligocene Transition (EOT, ~33.5 million years ago) – i.e., an interval in which an increase in diatom abundance and diversity is seen globally. Whereas this estimate appears to hold for the deep-sea realm, an examination of the stratigraphic distribution of diatomite – sediment composed of diatom valves – indicates that the neritic environments have witnessed high accumulation of biogenic silica long before the EOT. For instance, Paleocene-early Eocene (65–45 million years ago) successions of the Eurasian platform are largely composed of diatom-rich sediments, including diatomite. This implies an onset of high diatom production predating the EOT, which is consistent with the recently established link between elevated rates of terrestrial silicate weathering under the early Paleogene greenhouse climates and the resultant high supply of dissolved

silica to neritic waters. It also demonstrates the high significance of shallow marine sediments, rather than deep-sea records, for reconstructing the history of diatom ecological significance.

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EARLY EOCENE CHERT FORMATION IN THE NORTH ATLANTIC DRIVEN BY ELEVATED NERITIC DIATOM PRODUCTION

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Early deep sea drilling operations in the Atlantic both targeted and were greatly hindered by ubiquitous indurated siliceous sediments of early Paleogene age. It has been well documented that many of the strong seismic reflectors in the North Atlantic correspond to Eocene cherts and porcellanites, but these horizons continue to present a paleoceanographic puzzle. Although various biogenic and abiogenic processes have been invoked to explain their formation (including elevated rates of biosiliceous sedimentation, volcanism, and clay mineral-mediated precipitation), the source of silica required to produce such volumes of cristobalite/tridymite and quartz in early Eocene sediments in the Atlantic, has remained elusive. To this end, we have generated biogenic silica records from ODP Sites 1050, 1051 and 1053 on the Blake Nose spanning the early Paleocene-to-late Eocene interval, supplemented by bulk carbonate stable carbon isotope records and a characterization of siliceous microfossil assemblages from multiple sites in the North Atlantic Ocean. Building on an early hypothesis by Weaver and Wise in 1974, we propose prolonged elevated rates of shallow-water diatom production on the North American margin during the early Paleogene, combined with an intense lateral transport into the pelagic zone of the North Atlantic, as the source of silica for the formation of widespread diagenetically altered siliceous sediments in deeper waters. Neritic diatom production constitutes an overlooked link between elevated terrestrial silicate weathering rates associated with the early Paleogene greenhouse climates and deep-sea silica burial and subsequent

diagenesis. The scenario proposed here is simpler than previous hypotheses on chert/porcellanite occurrences in the early Paleogene, but also supported by a range of micropaleontological evidence and consistent with modeling results.

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EUGLENOID BLOOMS – CAUSES AND EFFECTS

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Among euglenophytes one can distinguish phototrophs, heterotrophs and mixotrophs. These multiple modes of nutrition have caused euglenoids to occupy almost all types of water habitats. Undoubtedly, their development is limited neither by high water nor air temperature ranges. When creating blooms, their propagation by cell division is rapid. Under favourable conditions their biomass increase is greater than other microalgae. Ability to produce temporal, reproductive and protective cysts also contributes to rapid bloom formations. Our observations prove that their features make them model organisms for observing environmental changes in small water bodies. So far, little attention has been given to the mechanisms of euglenoid blooms. During long-time research that had been carried out in Poland and Thailand, we studied shallow ponds that were ammonia nitrogen- and organic matter-loaded. During blooms water temperature and different physicochemical parameters were extremely high (e.g., temperatures up to 38.6°C; N-NH₄: 4.0–5.6 mg/L; BOD: 42.8–45.6 mg/L; conductivity: 0.7–0.9 mS/cm; pH: 8.4–9.4). We observed that *Euglena sanguinea* most frequently caused euglenoid blooms. This species possesses a high carotenoid content resulting in spectacular red blooms. In small ponds, both in temperate and tropical climates, we often noted blooms caused by *Euglena viridis*, *E. geniculata*, *Euglenaria caudata*, *Monomorphina pyrum*, *Trachelomonas volvocinopsis*, and *T. volvocina*. We also noted in tropical zones that the blooms were much denser, often forming thick and solid film on the water surface causing totally anoxic conditions. Our results have shown that these microorganisms can be used as indicators of an increase of organic and inorganic

pollutant concentration in high-temperature weather conditions. These bioindicators might be especially important in changes of water bodies during the global warming process. Additionally, they might be used as bioremediation organisms, since they are able to incorporate chemical compounds rapidly in their biomass.

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THE FUTURE FOR BALTIC CYANOBACTERIA?

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Around 85 million people live in the catchment area of the Baltic Sea, which subjects it to a range of environmental pressures and combined with low alkalinity, variable salinity and limited water exchange, the Baltic Sea may be less resilient to future stressors such as climate change. In the Baltic Proper, there is a succession of blooms, within the microplankton community, from diatoms and dinoflagellates in the early spring to cyanobacteria during summer and a second diatom and dinoflagellate bloom in the autumn. The cyanobacteria of the Baltic Proper are dominated by *Aphanizomenon* sp. and *Nodularia spumigena*. *Dolichospermum* spp. is less abundant. The effects of climate change were tested on a natural microplankton community, as well as on isolated cyanobacteria species from the Baltic Sea. To simulate effects of climate change, the temperature was increased from 12°C to 16°C, salinity decreased from 6–7 to 3–4 and atmospheric pCO₂-levels were increased from 380 ppm to 960 ppm. The biovolume of *Aphanizomenon* sp. and *N. spumigena* increased when temperature was increased by 4°C. When salinity was decreased by three units, both the growth and photosynthetic

activity of *N. spumigena* were reduced while *Aphanizomenon* sp. was unaffected, and the growth of *Dolichospermum* spp. was increased. Furthermore, present-day salinities were beneficial, in terms of increased biovolumes, of diatoms, dinoflagellates and ciliates, compared to reduced future salinity. Increased atmospheric $p\text{CO}_2$ had no effect on any of the species in the microplankton community. An increase of cyanobacteria blooms may open up to the possibility to grow and/or harvest these species as a source of biofuel or fatty acids (FA). *Dolichospermum* sp. yielded higher total FA content per biovolume, compared to the other two cyanobacteria species in phosphorus-depleted medium and *Aphanizomenon* sp. in nitrogen-depleted medium. This indicates a possible future market for biofuel and FA technologies.

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ICE DIATOMS – STRESSED OR NOT STRESSED?

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Benthic diatoms and sea ice diatoms are a vital part of the primary production in polar regions and provide a substantial carbon source for higher trophic levels. In a series of studies we investigated how environmental stressors, such as increased temperature and elevated CO_2 , affect microbial physiology and community structure in polar areas. The ecophysiology of sea ice microorganisms was addressed in laboratory experiments and in field measurements. In brief, relatively small changes in temperature had considerable effects on the physiology of sea ice diatoms, and

indirectly affected the structure of sea ice bacterial communities. Increasing temperature (climatic and seasonal scales) positively affected the growth and primary productivity of two sea ice diatom species, and negatively affected the taxonomic richness and diversity of sea ice bacterial communities. The sea ice diatoms were quite tolerant to changes in pH and partial pressure of CO_2 ($p\text{CO}_2$) in terms of growth, probably due to the fact that they grow in an environment with large seasonal variations in the carbonate system. However, increased $p\text{CO}_2$ resulted in other cellular changes that may have important ecological consequences, such as cellular stoichiometry. This includes changes in fatty acid composition and dissolved organic carbon exudation, which are important components in food webs and biogeochemistry in marine ecosystems.

Although most studies on marine organisms have focused on short-term responses to increased $p\text{CO}_2$, acclimation and adaptation are key components in order to identify the consequences of climate change in biological systems. The physiological responses to long-term acclimation to high $p\text{CO}_2$ were investigated in the sea ice diatom *Nitzschia lecontei*. After long-term acclimation (194 days), a small reduction in growth was detected at high $p\text{CO}_2$. Previous short-term experiments have failed to detect altered growth in *N. lecontei* at high $p\text{CO}_2$, which illustrates the importance of experimental duration in ocean acidification studies.

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EFFECTS OF CULTURAL CONDITIONS ON ARCHEOSPORES FORMATION OF PYROPIA YEZOENSIS AND THE TRANSCRIPTOME ANALYSIS

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Archeospore is a kind of asexual reproduction strategy for the genus *Pyropia*. It is very important for *Pyropia yezoensis* to improve the efficiency of artificial breeding if we can find suitable methods to increase the archeospores production. In this experiment, firstly we explored the effects of different environmental factors, such as temperature, light, salinity, aeration and so on, on archeospores formation from the blades of *P. yezoensis*. Our results reconfirmed that *P. yezoensis* had the highest productivity

of archeospores on 15°C and 9L/15D which means that short light rhythm would improve its formation as reported early. It also showed that the light intensity did not have a significant effect on archeospores formation, but higher light could maintain the laver in better growing condition. The most important result was that we found that the temperature alteration had great effect on the archeospores formation so the number of released archeospore for all the experimental groups with different temperature at light and dark period was higher than the control group with stable temperature. The best result was the group cultured under 15°C at light period and reduced to 10°C at dark period, which somewhat mimicked the natural environment. Then the transcriptomes of samples from day 0, 2, 4 and 6 cultured with the optimal condition were sequenced and compared for the analysis of relative genes involved in this process.

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A NOVEL APPROACH TO STUDY THE METABOLOMICS OF A FACULTATIVE MICROALGAE *CHLORELLA SOROKINIANA* WITH TRANSITION IN TROPHIC MODE BY TWO STAGE OPERATION IN COMPARISON WITH IN SILICO STUDIES

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Many studies have reported that heterotrophic and mixotrophic growth of various microalgal species yields greater biomass, lipid content and high-value-added bioactive compounds as byproduct (pigments) when compared to photoautotrophic cultivation. Microalgae have become an important commercial source of high value added products, and much research has been focused on lipids. Microalgae also produce metabolites such as carotenoids: β -carotene, lutein, zeaxanthin, zeaxanthin and astaxanthin. These bioactive compounds have high commercial value as nutraceuticals, cosmetics and in the food industries.

This study explores the possibility of enhancing the natural ability of the microalgal metabolomics of *Chlorella sorokiniana*, a facultative microalgae, by the transition of trophic mode using a two stage operation. Here the two stage operation limits nitrogen, phosphorus and light intensity in the stress phase, whereas carbon and bicarbonate were supplemented in both the growth and stress phases. This study displayed intriguing results, in that biomass

and chlorophyll production were 4 and 12 fold higher, respectively, compared to autotrophic to mixotrophic with 2.8 g L⁻¹ and 11.85 g L⁻¹, 4.2 μ g mL⁻¹ and 62.9 μ g mL⁻¹ respectively. Additionally in the present work, microalgae are selected as a natural source of bioactive compounds as a byproduct (apart from lipids and biomass). Interestingly the production of lutein was reported in a higher amount in mixotrophy (7 mg g⁻¹) than in autotrophy and heterotrophy. There were huge variations observed in metabolomics from this transition mode of operation from autotrophy to heterotrophy, heterotrophy to autotrophy and even in heterotrophy in comparison with autotrophy mode of nutrition. This demonstrates the potential advantage of the overall economics for the production of biodiesel and value-added biomolecules from microalgae.

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SILICIFIED BOLIDOPHYTES (PARMALES, BOLIDOPHYCEAE) IS NOT MONOPHYLY AS EVIDENCED BY THE PHYLOGENETIC CHARACTERIZATION OF *TRIPARMA RETINERVIS*

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The bolidophytes, a tiny phytoplankton lineage containing one order Parmales, includes two distinct morphotypes, namely, the cells covered with silicified plates (parma-type) and naked flagellated types (bolido-type). Because the nature of the siliceous cell wall found in the parma-type resembles that of diatoms, a sister lineage to the bolidophytes, a better understanding of the phylogeny of the parma-type would shed new insight into the origin and evolution of the silicification of the cell wall. Among 23 parma-type taxa described so far we had already established strains belonging to four taxa which were monophyletic either in 18S rDNA or rbcL tree. In this study observation and phylogenetic analysis were undertaken with newly established parma-type strains. Morphology of two new strains was well-fit to *Triparma retinervis* with characteristic convex shield plates, non-keeled dorsal plate and fine venation on all the plates. In 18S rDNA or rbcL trees *T. retinervis*

was distinct from the clade for four parma-type taxa, being sister to *T. pacifica* (bolido-type; formerly classified as *Bolidomonas pacifica*) making the parma-type strains paraphyletic. Hypothesis testing using a constrained topology rejected the monophyly of the parma-type strains. Furthermore, apart from *T. retinervis*, our newly established collection included strains with a notable morphology: the most striking feature was cells covered with large number of silicified scales which were homogeneous in shape (circular) and small in size (ca. 200–500 nm in diameter). Therefore, the appearance of the cells was reminiscent of coccolithophorids instead of parma-type cells covered with heterogeneous and relatively larger plates. Although our preliminary phylogenetic analysis clearly placed the strains within the bolidophytes clade, their phylogenetic positions was not fully resolved, hampering further discussion on character evolution and origin of silicified cell wall etc at this stage. Our result implies the presence of more yet undiscovered diversity of the bolidophytes than previously thought.

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DISCOVERY OF A KLEPTOPLASTIDIC TERTIARY PLASTID IN A DINOFLAGELLATE

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Some dinoflagellates have replaced their secondary plastids of red algal origin with other microalgae. “Dinotoms” are one example, as they possess tertiary diatom-endosymbionts. These endosymbionts represent an intermediate stage on the way to plastid establishment, because the endosymbiotic diatoms (ESDs) still possess their nuclei, mitochondria and ribosomes in addition to their plastids, while these organelles are separated from host cytosol by a single membrane. On the other hand, although the endosymbionts may appear to have retained their structural independence, they permanently remain inside the host cell and show a synchronized cell division.

Here we demonstrate that the ESD of the dinoflagellate *Durinskia capensis* shows a similar behavior to temporary endosymbionts known as kleptoplastids. The ESDs in *D. capensis* gradually lose their plastids and nuclei during two months under starving conditions. The nuclei disappeared earlier than the plastids themselves, which is supported by PCR results from cDNA indicating that psbA

and 16S rDNA genes in ESD plastids are still expressed strongly after five weeks of cultivation, while there is less detectable gene expression of 18S rDNA in the ESDs at same time. Sequencing the *rbcL* gene of the ESD plastids, collected in 2007, 2013, and 2017, revealed that the same ESD can be always identified, which is closely related to *Nitzschia draveillensis*. This indicates that *D. capensis* established a species-specific relationship with a single diatom species. Most interestingly, *D. capensis* is very closely related to another dinotom, *D. kwazulunatalensis*. Both species are very similar with respect to ecological behavior, habitat, morphology, and nucleotide sequence. The permanent ESD of *D. kwazulunatalensis* is related to *Nitzschia fonticola*, so possibly after the *Durinskia* species separated, *D. capensis* may have changed their ESD specificity to *N. draveillensis*, and back to a temporary plastid stage.

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PHYLOGEOGRAPHIC PATTERNS INDICATE CRYPTIC SPECIES DIVERSITY IN THE RED ALGAE GLOIOPELTIS FURCATA (GIGARTINALES) IN THE NORTHERN PACIFIC REGION

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Recent molecular analyses have demonstrated that many widespread species consist of species complexes with high genetic diversity. The red algae *Gloiopeltis furcata* is a suitable candidate species for studying cryptic diversity because of its broad distribution in the northern Pacific Ocean. To examine the genetic diversity within the species in relation to current distribution, we analyzed a total of 108 COI-5P and 110 *rbcL* sequences from *G. furcata* specimens collected in Korea, Japan, Russia and Canada. Phylogenetic trees and haplotype analyses of COI-5P and *rbcL* revealed high cryptic diversity comprising five distinct genetic groups within *G. furcata*. The five genetic groups were identified as 1) Korea/Japan/Canada/Russia population, 2 & 3) Korea/Japan population, 4) Japanese

population, and 5) Korean population. Our results show the highly complicated genetic structure in specimens from Korea and Japan and extremely low haplotype diversity in Canada compared to the western Pacific. The presence of disconnected groups in *G. furcata* represents several novel species, highlighting the importance of phylogeographic approaches for discovering cryptic algal biodiversity.

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OVEREXPRESSION OF THE SMALL HEAT SHOCK PROTEIN, PtsHSP19.3 FROM MARINE RED ALGAE, PYROPIA TENERA (BANGIALES, RHODOPHYTA), ENHANCE ABIOTIC STRESS TOLERANCE IN CHLAMYDOMONAS

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Pyropia tenera, a marine red alga, belongs to the order Bangiales (Rhodophytes) and is one of the most valuable and cultivated *Pyropia* species. Water temperature is one of the major factors affecting the growth and life cycle in *Pyropia*. We analyzed the transcriptome from gametophytes of *P. tenera* under normal- and high-temperature conditions and identified four small heat-shock proteins (sHSPs). Among them, we cloned a cDNA of the *PtsHSP19.3* that encodes a polypeptide with 173 amino acid residues, 19.3 kDa and pI of 5.4. The *PtsHSP19.3* gene responded to high temperature but only slightly or not at all to desiccation, freezing or high salt conditions. When the *PtsHSP19.3* gene was overexpressed in *Chlamydomonas reinhardtii*, the transformed *Chlamydomonas* lines showed a much higher growth rate than that of the control cells under heat-stress conditions. The transformed cells also grew better than the control cells on medium containing high salt or H₂O₂. However, the transformed *Chlamydomonas* did not show tolerance to osmotic stress induced by mannitol or low temperature. When the *PtsHSP19.3* was fused to

GFP and introduced into tobacco protoplast, fluorescence was detected at several spots. These results indicate that *PtsHSP19.3* may form super-molecular assemblies or small cellular granules such as stress granules, and is involved in tolerance to heat stress.

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EFFECTS OF SEA LETTUCE (*ULVA RIGIDA* C. AGARDH, 1823) AS FEED SUPPLEMENTS IN DIET ON GROWTH PERFORMANCE AND FEED UTILIZATION OF SPOTTED SCAT (*SCATOPHAGUS ARGUS* LINNAEUS, 1766)

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The effects of different dietary percentages of algae, sea lettuce (*Ulva rigida*) on average weight (AW), weight gain (WG), specific growth rate (SGR), rate of feed intake (FI), feed conversion ratio (FCR), survival rate (SR), protein efficiency ratio (PER) and feed conversion efficiency (FCE) of spotted scat (*Scatophagus argus*, 0.49 ± 0.11 g initial body weight) were investigated. Seven isonitrogenous and isocaloric diets but different percentages of sea lettuce 0 (control), 5, 10, 15, 20, 25 and 30% were used. Diets were fed to triplicate groups of fish two times a day to apparent satiation for 10 weeks. Results showed that fish fed 5 and 10% seaweed had the highest mean AW, WG and SGR ($P < 0.05$). Spotted scat fed the control diet and 5% *U. rigida* showed poorer FI than fish fed diets containing 10–30% ($P < 0.05$) of FCE but higher than the fish fed control diet and 30% of *U. rigida*.

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MULTIGENE, ORGANELLAR AND NUCLEAR GENOME DATA PROVIDE THE BIG PICTURE OF RED ALGAL EVOLUTION

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Red algae (Rhodophyta) include more than 7,000 species that thrive in marine and freshwater habitats. Red algae play a critical role in the eukaryote tree of life as the donor, through secondary endosymbiosis, of the plastid that subsequently gave rise to chlorophyll-*c* containing groups such as diatoms, dinoflagellates, haptophytes, and cryptophytes. In order to increase our understanding of the diversity and evolution of red algae, to make a linkage to the ‘Assembling the Tree of Life’ we conducted taxon-rich multi-gene phylogeny of red algae. We assembled ten genes (plastid *rbcL*, *psaA*, *psaB*, *psbA*, and UPA, mitochondrial *cox1* and *cob*, nuclear EF2, SSU and LSU rDNA) from 42 orders 570 taxa. Our best phylogeny based on individual and combined dataset provides highly resolved relationships in most taxonomic ranks. Phylogenies using plastid, mitochondrial, and nuclear genome data were also compared. Based on the phylogeny we suggest a new red algal taxonomic system. In addition, we report the 92.1 Mbp of the complete nuclear genome of the agarophyte *Gracilariopsis chorda* that encodes a total of 10,806 predicted proteins. Comparative genomics demonstrate that red algae have followed a unique evolutionary trajectory distinct from other multicellular eukaryotic lineages.

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METHANOLIC EXTRACT OF SARGASSUM CRISTAEOFOLIUM INDUCE THE APOPTOSIS OF HUMAN MAMMARY CARCINOMA CELLS

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The aim of this study was to investigate the role of methanolic extract of *Sargassum cristaefolium* on the apoptosis process in breast cancer cell line MCF7. MCF7 cell line was treated with different concentrations of methanolic extract of *Sargassum cristaefolium*: 7.14 ppm (<IC50), 14.28 ppm (=IC50), 21.42 ppm (>IC50) with different incubation times (3, 6, 9, 12, 24 h). It was found that an increase in the concentration of methanolic extract and incubation time has an impact on the induction of caspase 3 activation and apoptosis process on MCF7 cell line. Flow cytometry analysis showed that the incubation of MCF7 cell line in 14.28 ppm of *Sargassum* methanolic extract could inhibit about 30% of MCF7 cell line development.

EUSTIGMATOPHYTE ALGAE HOST AND EXCHANGE GENES WITH BACTERIA REPRESENTING A NEW GENUS OF RICKETTSIACEAE

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ALFF Rickettsiales are a group of obligate intracellular bacteria originally found in terrestrial animals, but more recently recognized as widespread endosymbionts of various aquatic hosts, particularly diverse protists. One rickettsial lineage was detected in several green algae, but reports on rickettsial endosymbionts in other algal groups are missing. We discovered that several phylogenetically diverse eustigmatophyte algae are infected by a new member of the family Rickettsiaceae, denoted *Candidatus Phycorickettsia trachydisci* (further referred to as *Phycorickettsia*). Fluorescence in situ hybridization and transmission electron microscopy demonstrated the occurrence of multiple endosymbionts in the cytoplasm of infected algal cells. We sequenced the genome of the endosymbiont in *Trachydiscus minutus* CCALA 838 and found that its size (1.47 Mbp), GC content (34%), and number of predicted genes (~1,250)

are in the range typical for related bacteria. A phylogenomic analysis resolved *Phycorickettsia* as a separate lineage of Rickettsiaceae, sister to previously sequenced members of the family. Comparative analyses of the gene repertoire revealed a number of noteworthy features, including the absence of genes for the flagellum, respiratory complexes III and IV, and the haem biosynthesis pathways. On the other hand, *Phycorickettsia* exhibits a complete pentose phosphate pathway (so far not known from Rickettsiaceae) and a highly expanded set of ankyrin-repeat proteins (putative effectors delivered to the host cell by the T4SS secretion system). Most interesting is the presence in *Phycorickettsia* of a six-gene cluster that corresponds to the *ebo* operon we recently reported from the plastid genomes of two eustigmatophytes and various bacteria. Strikingly, the operons from *Phycorickettsia* and the eustigmatophyte plastomes are specifically related, indicating genetic exchange between the endosymbiont and host lineages. We hypothesize that *Phycorickettsia* is a long-term eustigmatophyte partner and that the interaction is partly dependent on the function of the *ebo* operon.

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DIATOMS AS INDICATORS OF ENVIRONMENTAL HISTORY AND HUMAN IMPACTS DURING THE LATE HOLOCENE AT QARUN LAKE, FAIYUM OASIS, EGYPT

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A detailed diatom study of three cores recovered from Qarun Lake at the north of Faiyum Oasis was performed to reconstruct the environmental history and human impacts during the late Holocene. Diatom analysis of the studied core sediments revealed an interesting history of changes in lake water levels and a series of fluctuations between fresh and brackish episodes. The distribution pattern of the recognized diatoms and multivariate statistical analyses distinguished fourteen diatom ecological groups, which reflect distinctive environmental conditions that mainly related to climatic changes and anthropogenic effects during the late Holocene. The results denote that the late Holocene hydrological system of the Faiyum Oasis was active, variable and mainly affected by human activities through different civilizations from the New Kingdom to the present. The diatom record revealed five phases of lake development, which were represented by oscillations between freshwater and

relatively deep lake during humid-wet periods, and brackish water conditions that prevailed in warm-arid episodes. Abrupt change in the Qarun Lake has occurred during the last century, since the lake has changed from highly brackish water to saline, which was characteristic for the last phase of lake evolution. The salt build-up in the lake is indicated by increasing abundance of mesohalobous and polyhalobous diatom taxa, which designate a striking rise in salinity of the lake due to a great reduction of fresh water from adjacent areas, as well as an increased evaporation process due to a warm dry climate. Increasing pollution and eutrophication of the Qarun Lake waters was also recorded in the uppermost sediments of the studied cores. Rising salinity and high nutrient loading due to human activities has allowed for the growth of the diatom community of the marine affinity in recent times.

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EVERYTHING'S DIFFERENT: RECONSTRUCTING METABOLIC PATHWAYS OF THE NON-PHOTOSYNTHETIC PLASTID OF EUGLENA LONGA

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Non-photosynthetic plastids (leucoplasts) appeared repeatedly in diverse eukaryotic lineages. The best-studied example is the apicoplast of the apicomplexan parasites (e.g. *Plasmodium falciparum*). We focused our research on *Euglena longa*, a non-photosynthetic species closely related to the model photosynthetic euglenophyte *Euglena gracilis*. At first we searched for pathways essential for the apicoplast, i.e. tetrapyrrole, isoprenoid, and fatty acid biosynthesis, but surprisingly only alternative non-plastidic pathways could be found in *E. longa*. However, acyl carrier protein has been retained in the plastid, apparently to serve only as a carrier of imported acyl chains in the synthesis of plastid glycerolipids, including monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG). We confirmed the presence of these lipids by mass-spectrometry and immunofluorescence labeling of DGDG visualized *E. longa* leucoplasts as small foci inside the cell. Linearized Calvin-Benson cycle (CBC), lacking phosphoglycerate kinase and glyceraldehyde-phosphate dehydrogenase, was also found to localize to the *E. longa* plastids. Retention of the ferredoxin/thioredoxin (Fd/Trx) system in the plastid suggests that redox-dependent regulation of the CBC (specifically of fructose-1,6-bisphosphatase and phosphoribulokinase preserving the critical cysteine residues) may take place in the *E. longa* plastid despite the absence of photosynthesis. That the Fd/Trx system may be functional is further supported by the plastid localization of the SUF system, i.e. a machinery of Fe-S clusters that are essential components of Fd and Fd:Trx reductase. Our results show that the leucoplast of *E. longa* is significantly different from the apicoplast and other non-photosynthetic plastids characterized to date, and thus document how different the outcomes of independent reductive evolution of an originally highly complex ancestral entity, i.e. a photosynthetic plastid, may be.

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NEW INSIGHTS INTO LIPID METABOLISM AND SIGNALING IN THE RED ALGA PORPHYRA UMBILICALIS

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Here we reconstruct the putative pathway of fatty acid and lipid biosynthesis in the red alga *Porphyra umbilicalis* based on genomic data. Our findings suggest that lipid biosynthesis in red algae is similar to that in vascular plants and green algae although not all genes have been transferred to the nuclear genome, yet. Pathways from the eukaryotic host and the presumed cyanobacterial endosymbiont are also present. De novo fatty acid biosynthesis from Acetyl-CoA in vascular plants involves two multimeric enzyme complexes: Acetyl-CoA carboxylase and Fatty Acid synthase. In contrast to members of the green lineage, three out of four subunits of the acetyl-CoA carboxylase are encoded in the plastid genome, not in the nucleus.

Red algae lack the complete plastid desaturation pathway, with exception of the phosphatidylglycerol-specific Δ^3 -desaturase FAD4, which indicates, that the predominant C₂₀ fatty acids are ER derived. This suggests an important role for ER to chloroplast lipid trafficking in *Porphyra*. In *Arabidopsis* four proteins play a role in chloroplast lipid import: TGD1, 2, 3 and 4.

In *Arabidopsis* TGD1, 2 and 3 resemble a bacterial type ABC transporter, located at the inner chloroplast envelope. TGD4 is part of an outer envelope complex. TGD4 was not found in *Porphyra*, but orthologues for TGD1, 2 and 3 were identified. Interestingly, TGD1 and 2 are encoded in the plastidial genome, while the gene for TGD3 was found in the nuclear genome.

Currently no experimental data are available regarding sphingolipid metabolism in red algae. We could identify all genes necessary for the synthesis of sphingosine, sphingosine-1-phosphate and ceramide. Also the synthesis of more complex glycosphingolipids seems possible.

Our data also suggest that *Porphyra* is capable of synthesis of the phosphoinositides PI4P and PI(4,5)P₂, but not PI(3,5)P₂, the latter being involved in establishment and orientation of the cell plate in mosses and angiosperms. But we identified a phospholipase C candidate, responsible for the synthesis of the signaling molecules DAG and IP₃ from PI(4,5)P₂.

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TRACKING CHANGES IN THE SPECIES RICHNESS AND BIODIVERSITY OF BENTHIC DIATOMS IN COASTAL WATERS (NORTHUMBRIA, UK)

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Although significant progress has been made in diatom taxonomy, the composition and structure of marine and brackish benthic communities remain poorly known. The heterogeneity of coastal habitats potentially supports an enormous diversity of benthic diatom taxa but they have been significantly affected by human activities since the Industrial Revolution began. To demonstrate changes that have taken place on the British coast, a floristic analysis of historical (XIX century, Atthey collection stored in Royal Botanic Garden Edinburgh) and modern samples from the Northumberland area (North East England) was conducted. The analysis of historical samples enabled identification of benthic diatom communities expected to develop under less impacted conditions. They were composed of up to three-fold lower number of taxa than contemporary communities. Distinct differences were documented between historical and modern diatom communities regarding composition (species loss), structure (a transformation of communities heavily dominated by a single species into ones with a comparable contribution of many taxa) and size of frustules (a decrease in average volume of community forming taxa). Several working hypotheses were proposed to address these findings, which point at e.g. organic matter contamination followed by changes in zoobenthic communities (pressure of 'overdeveloped' meiofauna, top-down effect) or depletion of silica in marine waters. This study yielded extremely valuable data but more work is needed to reinforce these preliminary observations with comparisons from other coasts.

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EFFECTS OF COPPER (II) CHLORIDE, GLYPHOSATE AND IONIC LIQUID ON MIXED ALGAL CULTURES

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Photoautotrophic microorganisms significantly contribute to primary production and nutrient cycling in aquatic ecosystems. Therefore, it is essential to estimate their sensitivity towards chemical substances introduced to waters either intentionally or accidentally. In the past the toxicity

of many widely applied substances was incorrectly assessed (e.g. some pesticides).

Usually, ecological tests are conducted on single strains. In our experiments we tested the effect of copper (II) chloride, glyphosate and ionic liquid ([BMIM]Cl), by exposing a mixture of three diatoms (*Bacillaria paxillifera*, *Navicula gregaria*, *Navicula perminuta*) isolated from the Gulf of Gdansk (southern Baltic Sea) to effective concentrations chosen based on literature data, including those representing maximum environmental concentrations. The seven-day experiments were sufficient to observe inhibitory effects caused by each tested chemical.

The only taxon in which cell number did not increase in the control solution was *N. perminuta*. It also was the taxon which was most tolerant of the tested substances and showed an almost five fold increase in the last day of the experiment in 0.85 g dm⁻³ glyphosate and a four fold increase in 0.0001 g dm⁻³ CuCl₂, as compared to the control. A high concentration of [BMIM]Cl of 0.175 g dm⁻³ inhibited the growth of *N. perminuta* by only 20%. The most sensitive taxon was *B. paxillifera* and its abundance was reduced to about 10% of the control population even in the lowest of tested concentrations. Its growth was stimulated only by [BMIM]Cl in 0.0175 g dm⁻³ concentration of ionic liquid. For *N. gregaria* its cell number was reduced to 20–30% of the control population in the highest tested concentrations and no stimulatory effect was observed.

For each tested chemical, slightly different reactions were observed in particular diatoms. Interactions among them were also important, this was most visible during the last day of the glyphosate-experiment when growth of *N. perminuta* was observed. The obtained data underpins the complexity of mechanisms shaping the composition and structure of natural diatom communities and how they can be altered by anthropogenic factors.

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PALAEOCEANOGRAPHIC IMPLICATIONS OF DIATOMACEOUS OOZE FROM THE MARIANA TRENCH

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Diatomaceous ooze sampled from the top of the gravity core retrieved from the Mariana Trench revealed large quantities of *Ethmodiscus rex* fragments and the tropical open ocean planktonic diatom taxa *Azpeitia nodulifera* and *Alveus marina*. Sub-surficial sediments from the ooze are interpreted to the age of MIS2 in the last glacial stage. The occurrence of *E. rex* suggests massive blooms in the Northwestern Pacific Ocean during the late Pleistocene and is a plausible link to palaeoceanographic and palaeoclimate changes related to strong flows of Antarctic Intermediate Water (AIW) during the last glacial stage. This water mass/current-transported dissolved silica reduced diatom dissolution. The northward flow of AIW was possibly related to surface current migration and a southward shift of the northwestern Pacific gyre to form oligotrophic conditions that triggered *E. rex* blooms. The *E. rex* ooze formation may support a blooming hypothesis, oligotrophic habitat and differentiated dissolution of diatom species.

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PHYLOGEOGRAPHIC DATA REVEALED SHALLOW GENETIC STRUCTURE IN THE WILD KELP *SACCHARINA JAPONICA* (LAMINARIALES, PHAEOPHYTA)

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Saccharina japonica is a commercially and ecologically important kelp species widely distributed along the coast of Japan Sea. Here, we used sequences of the mitochondrial COI and trnW-trnL to reconstruct the phylogeography and demographic history of wild *S. japonica* populations

on a large geographic scale. Diversity indices suggested that *S. japonica* populations along the coast of Hokkaido exhibited the highest genetic diversity. The coast of Hokkaido might be the origin and diversification center of *S. japonica*. Bayesian Analysis of Population Structure (BAPS) revealed four clusters in the kelp species (cluster 1: Hokkaido and South Korea; cluster 2: northwestern Hokkaido; cluster 3: Far Eastern Russia; cluster 4: China). The network inferred from concatenated data exhibited two shallow genealogies corresponding to two BAPS groups (cluster 2 and cluster 3). We did not detect gene flow between the two shallow genealogies, but populations within genealogy have asymmetric gene exchange. Gene exchange among *S. japonica* populations could be caused by anthropogenic interference and oceanographic regimes. Bayesian skyline plots and neutrality tests suggested that *S. japonica* experienced postglacial expansion around 10.45 ka. Postglacial expansions and gene exchange apparently led to more shared haplotypes and less differentiation that in turn led to the present shallow phylogeographical patterns in *S. japonica*.

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FINE-SCALE PHYTOPLANKTON DIVERSITY AND SEASONAL DYNAMICS IN THE SARGASSO SEA REVEALED BY A DECADE OF HIGH-RESOLUTION SAMPLING

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Marine phytoplankton represent a metabolically and phylogenetically diverse functional group, which is recognized for its important contributions to the global carbon cycle and ocean food web. In order to study this complex group at ecologically relevant levels of taxonomic resolution, we have developed a high throughput workflow that leverages the additional genetic information contained in longer-read sequence data. This method discriminates microbial types with unprecedented detail through the phylogenetic placement of plastid-encoded 16S ribosomal DNA amplicons in manually curated alignments to identify taxonomic associations without assigning operational taxonomic units. Previously, we have been able to resolve multiple clades within diverse phytoplankton genera with robust bootstrap

support. We have now applied this method to long-term oceanographic time-series data in the northwestern Sargasso Sea to resolve spatio-temporal distribution patterns of phytoplankton. The surface waters of the Bermuda Atlantic Time-series Study (BATS) station experience annual convective mixing, which brings nutrient-rich deep water to the surface. Combined with robust chemical and physical data collected at the time of sampling, this study provides new insights into the ecological niche space of highly resolved phytoplankton clades. These data provide valuable parameters for model inputs and generate hypotheses to pursue in the laboratory and through additional targeted fieldwork.

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NON-MONOPHYLY OF BOSTRYCHIA SIMPLICIUSCULA (CERAMIALES, RHODOPHYTA): MULTIPLE SPECIES WITH VERY SIMILAR MORPHOLOGIES, A REVISED TAXONOMY OF CRYPTIC SPECIES

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The discovery of a plethora of cryptic species in many algal groups has led to speculation as to why and has affected taxonomy, causing reluctance to give names to species that look identical. While this is defensible for monophyletic cryptic species complexes, both our understanding of similar morphologies (cryptis) and nomenclature is challenged when we encounter non-monophyletic 'cryptic' species. *Bostrychia simpliciuscula* is a wide ranging species in which multiple cryptic species are known. Our increased sampling shows that this species consists of four lineages that do not form a clade, but lineages are sister to species with different morphologies. Careful morphological examination shows that characters, especially branched monosiphonous laterals and rhizoids morphology in haptera, is able to distinguish these four lineages into two groups, that are still not monophyletic. The similar morphologies in these lineages could be due to convergence, not lack of developmental constraints or lack of time to diverge morphologically; or maintenance of a generalized body plan that lacks identifiable characters. These lineages appear to have specific

biogeographic patterns and these will be used to propose a new taxonomy. *B. simpliciuscula* is confined to the tropics. One of these lineages matches a previously described species, *B. tenuissima*, that was synonymized with *B. simpliciuscula* in cold temperate Australasia. Another lineage is found in Japan in which a previous name is also available, *B. hamana-tokadai*; the last lineage is found in central New South Wales, morphologically it resembles *B. tenuissima*, with which it overlaps in distribution around Sydney, and is named as a new species, *B. kingii* sp. nov.

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FIRST REPORT OF CAULERPA MACRODISCA (DECAISNE) WEBER-VAN BOSSE IN THE WEST COASTAL AREA OF SABAH, MALAYSIA

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The siphonous green macroalga *Caulerpa macrodisca* (Decaisne) Weber-van Bosse is reported to be widely distributed around South-west Asia (Sri Lanka), the South China Sea, South-east Asia (Indonesia, Philippines, Singapore and Vietnam) and the Pacific Islands (Samoan Archipelago). There was no formal report on the existence of *C. macrodisca* in Malaysia. This study reported the first occurrence of the green algae, *C. macrodisca* in the coastal area of Menumbok, Sabah, Malaysia. *C. macrodisca* was first discovered in March 2016 growing around the sea cage of a local farmer in Sikalong Village, Menumbok. In May and July 2016, *C. macrodisca* was searched for around the coastal area near the sea cage and was discovered at 1.4 to 2.7 m depth. A further scuba search was carried out in August 2016 and confirmed the occurrence of *C. macrodisca* near the area at similar depth. Most of the collected samples were large and reproductive and a few were small and young but precociously reproductive. The samples were collected in May, July and August 2016 for further analysis through morphology and genetic comparison. The collected specimens were confirmed as *C. macrodisca*. A newly generated 18S rRNA *C. macrodisca* gene sequence was deposited into the GenBank database with an accession number of KY387611.

**EXPLORING VALUABLE MICROALGAE
OF DIATOMS: A FUTURE PLAN
FOR DEVELOPMENT OF MICROALGAE
AT LSIH, BRAWIJAYA UNIVERSITY,
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Diatoms are one major group of microalgae in seas and oceans that accounts almost half of primary food production. They have been identified as a promising candidate in biotechnology and in producing a variety of bioactive compounds. Thousand species from hundreds genera are recorded in the world. Their potential applications in pharmaceuticals, biofuels, nutrient supplements and cosmetics have gained attention increasingly. They have important source of natural and bioactive compounds such as fatty acids, lipids and Triacylglycerols (TAGs) that can be used for sustainable production of biofuels, sterols and steroids, oxylipins, isoprenoids. Other benefits in wide area of pharmacognosy, biotechnology and environmental stimulants have been reported by many authors. The secondary metabolite contained in microalgae has been known have role for human health. The main photosynthetic pigments in diatoms are chlorophylls a and c, fucoxanthin, diadinoxanthin and diatoxanthin. One of diatoms, *Haslea ostrearia* has long been known for producing blue pigment marenine that presents different biological activities. BioEcotox Research Center at the Central Laboratory of Life Sciences (LSIH), Brawijaya University is developing culture plant for microalgae. Several species, including diatom from the genus *Haslea* are on the step of the Laboratory Scale culture. Future development has been programmed for the next ten years.

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