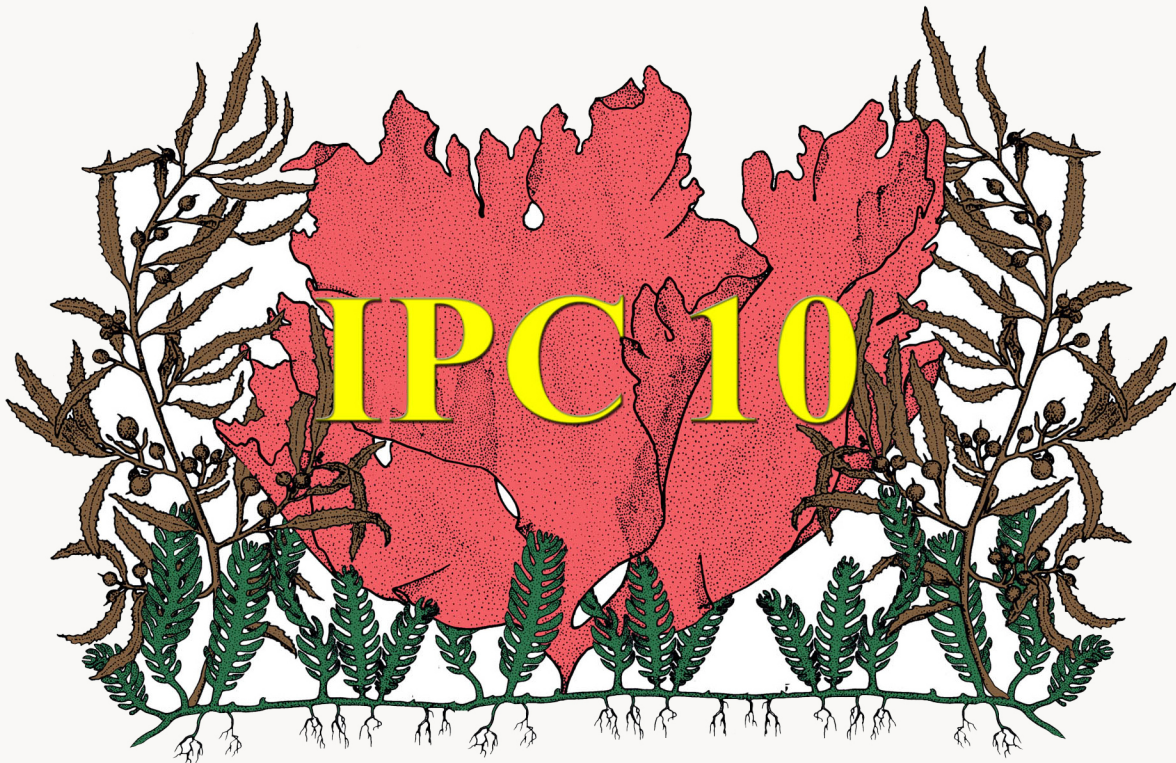


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**10TH INTERNATIONAL PHYCOLOGICAL CONGRESS
4-10 AUGUST 2013, ORLANDO, FLORIDA USA**

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 **International
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Society**

Edited by M. Dennis Hanisak, James A. Nienow & Akshinthala K. S. K. Prasad

Abstracts of papers to be presented at the
10th International Phycological Congress

Orlando, Florida, USA

4-10 August 2013

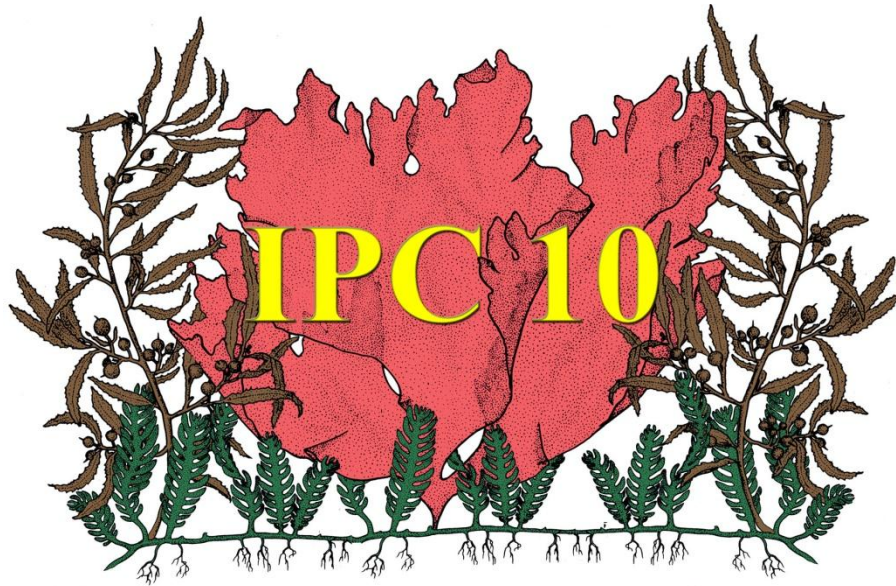
Held under the auspices of the International Phycological Society

Edited by: M. Dennis Hanisak, James A. Nienow & Akshinthala K. S. K. Prasad

Copies of the abstracts will be provided to registered delegates at the 10th International Phycological Congress. The abstracts are arranged in alphabetical order by first author, presenting authors are listed in bold.

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**10TH INTERNATIONAL PHYCOLOGICAL CONGRESS
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Description of the Congress Logo

The three macroalgae in the logo represent the three major taxa of eukaryotic macroalgae; all are native to Florida. The central position of *Halymenia floridana* J. Agardh [*Halimena floridana*] in the logo represents phycologists coming together in Florida. The other two algae represent the international nature of Phycology and the IPC. The alga forming the base of the logo is *Caulerpa mexicana* Sonder ex Kützing. While named for its type locality in the country of Mexico, it is found not only in Mexico and Florida, but also in warm waters of many other parts of the world. Bracketing the logo along both sides is *Sargassum natans* (Linnaeus) Gaillon, a pelagic macroalga found throughout the North Atlantic; as is the case with phycology, individuals of this species know no national boundaries. The images were modified from C. W. Schneider and R.B. Searles's 1991 monograph *Seaweeds of the Southeastern United States: Cape Hatteras to Cape Canaveral*, with their kind permission.

Charles D. Amsler and M. Dennis Hanisak
Committee, IPC10

Local Organizing

1

QUALITATIVE AND QUANTITATIVE ANALYSIS OF EXO-POLYSACCHARIDES FROM INDIGENOUS RHIZOSPHERIC CYANOBACTERIA

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Exo-polysaccharide (EPS) is an important trait of the majority of cyanobacteria that performs multiple functions. Physio-chemical analysis of EPS from locally isolated rhizospheric cyanobacterial strains and their comparison was the main aim of the current study. Eight cyanobacterial isolates, four unicellular and four filamentous, were used. Three different EPS fractions, i.e., released (REPS), loosely bound (LEPS) and closely bound (CEPS), were isolated separately from axenic cultures. Total protein and carbohydrate content was also estimated. Eight different monosaccharides were analysed in all EPS fractions using the HPAE-PAD technique. EPS layers outside the cyanobacterial cells were localized by confocal laser scanning microscopy (CLSM) using fluorescent dyes and lectins. Cyanobacterial isolates demonstrated a great variation in their EPS quantity and quality. CLSM revealed thick EPS increments on cyanobacterial strains, especially on *Chroococcidiopsis* strains (MMG-5 and MMG-6), whereas in filamentous strains released EPS was more visible. Quantitatively CEPS was the most abundant fraction constituting more than 50% of the total EPS of all strains. REPS contained the highest total carbohydrate content as compared to other fractions. Six monosaccharides, i.e. fucose, galactose, glucose, mannose, rhamnose, and xylose, were found to be the most commonly occurring residues in EPS of isolates. Fructose was found to be most common in *Chroococcidiopsis* strains. The EPS was found to be very diverse in nature and different fractions showed specific characteristics. This can be a source of interesting polysaccharides of desirable characteristics in biotechnology.

2

DIFFERENTIAL GENE REGULATION PATTERNS, DURING BLOOM AND NON-BLOOM TIME PERIODS, OF *ULVA RIGIDA*

IN NARRAGANSETT BAY (RHODE ISLAND, USA)

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The macroalga *Ulva rigida* develops into harmful blooms in shallow coastal systems worldwide, including Narragansett Bay, RI. These blooms have significant negative ecological and economic impacts on coastal communities. However, few data exist on mechanisms underlying functional genetics that link bloom dynamics to environmental changes, especially with respect to global warming. Individuals from pre-bloom, bloom, and post-bloom stages were collected during spring and summer 2012, along with relevant ecological distributional data. RNA was extracted from samples representing these three time periods (May, July and September 2012) and preliminary results using Real Time PCR showed differences in the expression of LhcSR, a gene that plays an important role in photo-protective mechanisms, with an increased gene expression during summer. We are preparing libraries to use an Illumina HiSeq transcriptome sequencing approach to characterizing and annotating the transcriptomes. Ultimately, we will compare the gene expression patterns found for *Ulva rigida* during bloom periods with those from non-bloom *Ulva rigida*. We anticipate that the transcriptomes will be highly similar among temperatures and that the critical regulators may be expressed at low levels.

3

STRUCTURAL EVOLUTION OF MITOCHONDRIAL GENOMES IN EARLY DIVERGING ALGAL LINEAGES

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Prasinophytes are a paraphyletic assemblage of green algae that include the earliest diverging lineages in Chlorophyta. These single-celled, planktonic organisms are found in marine and freshwater habitats worldwide. Lineages are recognized by characters

associated with scale morphology, flagellar apparatus, and cell division processes. Our ability to test hypotheses about early algal evolution is limited because relationships among these lineages are poorly understood. Seven distinct prasinophyte clades have been identified (Clades I-VII), and four completely sequenced mitochondrial genomes from Clades II, III, and V are available. We generated mitochondrial genomes from three additional lineages (Clade I, IV and VI) and increased taxon sampling in the Mamiellales (Clade II), the most diverse group of prasinophytes. Typically, green plant mitochondrial genomes do not contain an inverted repeat (IR); an architectural feature common in plastid genomes that minimally contains small and large subunit ribosomal genes. However, of the completed mitochondrial genomes sequenced across green plants, two prasinophyte mitochondrial genomes from the Mamiellales (Clade II) have been found to contain IRs. Interestingly, these mitochondrial IRs also include ribosomal genes. Newly sequenced mitochondrial genomes from Pyramimonadales (Clade I) and Chlorodendrales (Clade IV) also contain IRs, while those from Mamiellales (Clade II) and Prasinococcales (Clade VI) include members that have an IR and other members that do not, which illustrates the plasticity of this architectural feature in prasinophyte mitochondrial genomes. Inverted repeats have not been identified in mitochondrial genomes from representatives of Clades III and V, and mitochondrial genomes have not yet been sequenced from representatives of Clade VII. Details of genome architecture, gene content, and evolutionary relationships among prasinophyte lineages will be discussed.

4

CONSUMERS MEDIATE THE EFFECTS OF EXPERIMENTAL OCEAN ACIDIFICATION AND WARMING ON PRIMARY PRODUCERS

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We know little about the direct and indirect effects of ocean acidification, and likewise how these effects interact with other features of environmental change

such as warming and declining consumer pressure. In this study, we tested whether the presence of consumers influenced the interactive effects of ocean acidification and warming on benthic microalgae in a seagrass community mesocosm experiment. Net effects of acidification and warming on benthic microalgal biomass and production, as assessed by analysis of variance, were relatively weak regardless of grazer presence. However, partitioning these net effects into direct and indirect effects using structural equation modeling revealed several statistically strong relationships. Our analyses highlight that (i) indirect effects of climate change may be at least as strong as direct effects, (ii) grazers are crucial in mediating these effects and (iii) effects of ocean acidification may only be apparent through indirect effects, and in combination with other variables. These findings highlight the importance of experimental designs and statistical analyses that allow us to separate and quantify the direct and indirect effects of multiple climate variables on natural communities.

5

CYANOBACTERIA IN SOILS FROM A MOJAVE DESERT ECOSYSTEM

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The Fort Irwin National Training Center in the Mojave Desert of California contains soils that represent a diversity of disturbance regimes, including some soil sites that have been protected from anthropogenic disturbance for many years. Previous studies of the soils of this military installation discussed biogeography and ecology of the crusts but did not conduct detailed study of the species present. In this study, we selected 6 sites in and around the Fort Irwin Training Center to be the subject of intensive isolation efforts. These sites contained at least some development of microbiotic soil crust. Out of the more than 90 cyanobacterial isolates characterized, only 23 distinctive morphospecies were differentiated. Of these, only 13 were identified to a previously described species, the others being comparable to previously described aquatic species (cf.) or so dissimilar to described taxa that they were given numbers as identifiers. *Leptolyngbya* was the most species rich and also the most commonly isolated

genus. Twelve filamentous genera were found; no coccoid representatives occurred in our samples. All taxa are described and illustrated. This study is significant as it is the first since the 1960s to examine cyanobacterial taxonomy of Mojave Desert soils in a systematic fashion. Our unidentified taxa are very likely new species but will require molecular sequence analysis before they can be named.

6

CHEMICAL MEDIATION OF ANTARCTIC MACROALGAL-HERBIVORE INTERACTIONS

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Macroalgae dominate hard bottom areas along the Western Antarctic Peninsula to depths of up to 40 m or more. Most of the macroalgae are chemically defended from a variety of macro- and mesograzers but harbor very high densities of amphipod mesograzers. The amphipods benefit from living on the large, chemically-defended macroalgae because they gain refuge from fish which are their primary predators. Most amphipod species do not consume most of the macroalgal species, but are of benefit to the macroalgae by keeping them relatively clean of epiphytic microalgae and filamentous macroalgae. One amphipod species, however, does consume some of the chemically defended red algal species and is able to sequester algal metabolites for its own use as defenses against fish. Amphipods also appear to have selected for a relatively high incidence of filamentous algal endophytes in some of the larger macroalgae. These endophytes can be, but are not always, detrimental to the hosts.

7

CRYPTIC GENETIC DIVERSITY CORRELATES WITH SECONDARY METABOLITE DIVERSITY IN ANTARCTIC PLOCAMIUM CARTILAGINEUM

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Plocamium cartilagineum is known to harbor numerous secondary metabolites, particularly halogenated monoterpenes, which act as potent chemical defenses against herbivory. An Antarctic amphipod, *Paradexamine fissicauda*, is an exception to this as it readily consumes *P. cartilagineum*. No-choice and four-way choice bioassays conducted at various time of a single year and between years yielded varied though still significant *P. cartilagineum* feeding rates. Subsequently, collections within 3 km of Palmer Station (64° 46'S, 64° 08'W) were made to investigate genetic and secondary metabolite characters of individual *P. cartilagineum*. Three individuals were collected from seven different sites. Genetic analysis of *cox1* genes claded into two phylogroups and *rbcL* analysis grouped similarly with one exception. Neither gene differed enough to warrant species level-separation. Subsequent GC/MS QqQ analyses revealed five distinct chemogroups strongly correlating to the phylogroup and often to the collection location. Antarctic *Plocamium* may be undergoing cryptic speciation as is seen elsewhere, and its chemical diversity could be either a partial consequence or even a partial driver of this.

8

HETEROKONT ALGAE AND THEIR TREE OF LIFE

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The heterokont algae consist of approximately 16 classes if diatoms are classified as one class. Heterokont algae contain more species than other algal groups, largely because the diatoms are speciose. A complete phylogeny of the group has been lacking. We used a six-gene analysis (nuclear encoded SSU rRNA; plastid encoded *rbcL*, *psaA*, *psaB*, *psbA*, *psbC*) with a subset of 130 taxa representing all 16 heterokont classes to infer a phylogenetic tree. Three major clades were recovered, referred here to as SI, SII and SIII. The classes Aurearenophyceae, Chrysomerothyceae,

Phaeophyceae, Raphidophyceae, Schizocladiphyceae, Phaeothamniophyceae, and Xanthophyceae formed the SI clade. The Chrysophyceae, Eustigmatophyceae, Pinguiphycidae, Synchromophyceae and Synurophyceae formed the SII clade. The Bacillariophyceae, Bolidophyceae, Dictyochophyceae and Pelagophyceae formed the SIII clade. The approximately unbiased test rejected alternative hypotheses that forced each class into either of the other two clades. Existing morphological and biochemical data were consistent with the molecular phylogenetic tree, especially for the SIII clade. The clades could be interpreted as three divisions, the Phaeophyta, Chrysophyta and Bacillariophyta, respectively.

9

SYSTEMATIC REASSESSMENT OF *SARGASSUM* (FUCALES, PHAEOPHYCEAE) SPECIES FROM THE GULF OF CALIFORNIA, MEXICO

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Sargassum is an ecologically and economically important brown alga, and it is considered one of the most difficult phaeophycean genera in taxonomy. In the Gulf of California in Mexico, 42 specific and infraspecific names are to date applied and at least two morphological studies have been done, but species boundaries and the use of names remain unclear. Morphological characteristics, along with ITS, *cox1* and *cox3* sequences, were analyzed from specimens collected in the Gulf of California. In all three gene data, we confirmed *S. herporhizum*, *S. horridum*, *S. johnstonii*, *S. lapazeanum*, and *S. sinicola*. Molecular analysis revealed that the *Sargassum* species in the Gulf of California are clearly separated from those in other regions, indicating high endemism of the *Sargassum* species in this region. Two geographical origins of the *Sargassum* species are proposed: one group related to those from northern hemisphere likely dispersed into the region via the protogulf; the other group, *S. sinicola*, likely entered the Gulf of California when the southern end opened.

10

ASEXUAL PROPAGATION IN *SARGASSUM POLYCYSTUM*

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Sargassum polycystum is a widely distributed species. It is particularly common in Southeast Asia region where it is characterized by having a ramifying holdfast. It has been suggested that this species can propagate asexually using this ramifying holdfast but concrete experimental evidence is not available. In the field, the ramifying holdfast has seldom been observed to give rise to new individuals. We have cultured the ramifying holdfasts of this species and confirmed their ability to generate new thalli. Specifically, clean fragments of ramifying branches of the holdfast were grown under laboratory conditions. New ramifying branches from each fragment developed over a two month period. Thereafter, as soon as these bushy holdfast fragments were tied down on rocks, vertical upright axes developed within a week. Eventually, new caudal leaves also developed and the formation of a new young individual was complete. As many as 10 individual axes can emerge out of a 6 cm long fragment, suggesting the great potential of these holdfast fragments in developing new individuals. New sexual recruits from *Sargassum* are common, but only very few could successfully develop into adult individuals. The ability of ramifying holdfast fragments to generate new individuals could therefore serve as an effective alternative way to propagate the population and to pre-empt the space. It could also contribute to the dispersal of the species.

11

MICROALGAL BIODIVERSITY OF SPHAGNUM BOGS ALTERED BY HUMAN EXTRACTION ACTIVITIES IN SOUTHERN CHILE

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Sphagnum bogs are acidic, nutrient deprived, carbon sink ecosystems with a high environmental value.

However, because of expansion of cities around them, the commercial value of the moss together with its non-regulated extraction is having an impact on microbial biodiversity not yet reported in Chile. We measured and compared microbial biodiversity in samples of sphagnum from light extraction sites with sphagnum taken from heavy extraction sites from Puerto Montt and Chiloe. The total number of species recorded was 23 for all sites; two species (8.7%) were present in all sites, and ten species (43.5%) were found in just one type of site (light or heavy extraction mode). The site with highest diversity value (Shannon-Wiener H Index=1.95) was found in Chiloe from a *S. magellanicum* bog with a light extraction mode; the lowest H=0.67 was found in Puerto Montt from a *S. magellanicum* bog with a heavy extraction mode. *Nostoc* sp. was absent from this last site and very common in the first. We suggest that extraction activities rather than geographical differences or city population numbers are having an impact in the biodiversity of microalgae on sphagnum bogs.

12

COMPARATIVE TRANSCRIPTOMICS IN DIATOMS: USING A PHYLOGENETIC FRAMEWORK TO IDENTIFY GENES INVOLVED IN THE EXTRACELLULAR POLYMERIC SUBSTANCE-SECRETION PATHWAYS

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We are coming to understand that the ecological importance of diatoms is not limited to primary productivity, as many diatoms produce extracellular polymeric substances (EPS), which are vital components in algal and bacterial “biofilms.” While great effort has been made to chemically identify the types of molecules and polymers used to create and modify diatom EPS there is still much about the process we do not know. Rather than studying this process chemically, we have elected to search for the genes involved in EPS production and secretion. We assembled transcriptomes from a number of EPS-producing diatoms and diatoms which do not. In an attempt to limit the differences to EPS-related transcripts, the taxa were selected in a phylogenetic framework, where EPS-producing taxa were closely-

related to taxa which did not produce EPS. The resulting pool of transcripts was then compared to two annotated diatom genomes and sorted by function, looking specifically for genes related to secretion or polysaccharide assembly. These markers, considered possible genes related to EPS-production in diatoms, will be presented.

13

STATUS AND TRENDS IN THE PHYCOCOLLOID INDUSTRY – PRODUCTS, SEAWEEDS AND R&D OPPORTUNITIES

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Though the phycocolloid industry is quite established, it is constantly changing to meet challenges such as competition from other gums, growing demands from developing markets and socio-economic and environmental impacts on seaweed supplies. A brief overview is provided, trends are discussed and opportunities for research on commercial seaweeds are presented.

14

NON-NATIVE *BRYOPSIS MAXIMA* (ULVOPHYCEAE, CHLOROPHYTA) INTRODUCTION TO LONG ISLAND SOUND

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Introductions of non-indigenous marine species (NIS) have increased exponentially in marine ecosystems and pose significant threats to biodiversity by altering local community composition and ecosystem function. New England is particularly vulnerable to the introduction of exotic marine species as many species are at the limits of their distributional ranges. The region is also subjected to a high volume of ship traffic (both commercial and recreational) and shellfish aquaculture providing the mechanism to transport new species into the area. Furthermore, climate change may facilitate the establishment of NIS in New England. The chlorophyte *Bryopsis maxima* Okamura, is a large

pseudo-perennial macroalga, native to the western Pacific. This weedy species blooms in the spring and forms great masses growing on solid substrate compared to the modest growth of *B. plumosa* and *B. hypnoides*, the two native species commonly found in LIS. A robust population of an unknown species of *Bryopsis* was found in Waterford, CT, and Queens, NY. Our morphological and molecular analyses based on the psb gene of the unknown *Bryopsis* species have identified it as *B. maxima*, a native of Japan that has already been found in Virginia. From our initial observations, it appears to grow well under wide range of temperature, salinity and nutrient conditions. The thallus size (> 15 cm) and its occurrence in two very different habitats within the LIS estuary make this NIS of major concern. *Bryopsis maxima* has not yet expanded to inhabit all of LIS, and the rate and extent to which this will occur is unknown.

15

EFFICIENT OF SACCHARIFICATION OF *UNDARIA* SP. BIOMASS WITH GAMMA IRRADIATION

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Consumption of fossil fuel increased rapidly during the age of global industrialization. High energy consumption caused environmental problems, such as global warming, and it is also causing the exhaustion of fossil fuel. A potential solution to these worldwide problems is the development of alternative energy from renewable biomass. Approximately 20-30% of marine algae biomass is made up of cellulose or starch, and polysaccharides comprised with glucose, xylose, and galactose. Brown algae, also known as marine macroalgae, contain up to 67% carbohydrates by dry weight, including materials such as alginate, laminarin, and mannitol. Marine algae are an abundant, year-round source of carbohydrates. Moreover, algal cells can be harvested within a short span of time as compared to other land feedstocks, and hence they can meet the increasing demand of feedstocks for bioethanol production. However, the structures of polysaccharides from algae vary significantly, and the saccharification yield is not sufficient for economical production of bioethanol. Therefore, in this study,

changes in the reducing sugar of algae biomass caused by gamma irradiation, and the combined effect of irradiation and acid hydrolysis of marine algae for improving saccharification, were investigated.

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A STUDY OF MICROALGAL SYMBIOTIC COMMUNITIES WITH THE AIM TO INCREASE BIOMASS AND BIODIESEL PRODUCTION

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Microalgae show great promise as feedstock for production of biodiesel. Many species exhibit fast growth rates, reach high cell densities and contain large amounts of lipid. The aim of this study is to assess the importance of the symbiosis between microalgae and bacteria in order to increase both growth rate and biomass production. These factors are important to optimize microalgal derived biodiesel production. Three species of bacteria, *Halomonas* sp., *Marinobacter* sp. and *Pelagibaca* sp., have been isolated from a monoculture of the chlorophyte *Dunaliella salina*. They all benefit the growth of the microalgae. *Halomonas* sp. has significant effect even at low concentration. As microalgae adapted to regional conditions may become important in microalgal biodiesel production, monocultures of both microalgae and bacteria have been established from local freshwater samples. The importance of symbiotic bacteria on the growth of these microalgae is currently being assessed.

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STUDIES IN DIVERSITY, ECOLOGY, AND MOLECULAR GENETICS (AFLP) OF CHAROPHYTES IN ISRAEL

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Charophyte flora of Israel, presently the most representative for the Eastern Mediterranean, has been occasionally studied since 1970 and more consistently in 2001-2012, revealing a diversity of about 20 species

from 45 localities over six ecological regions, including the Arava Valley and Negev Desert. Hypsometrically, the lowermost locality occurs at 347 m b.s.l. in the Dead Sea rift. One genus and two species were found for the first time in Israel. Ecological characteristics and aquatic communities have been assessed for each sampling site. The major influence on charophyte distribution is climate, with sharp North – South gradients of temperature and humidity. Our preliminary results of molecular genetic studies using AFLP analysis show the differences in %P level related to extreme environmental conditions in the Negev Desert compared to the Northern Israel, a typically Mediterranean region. The candidate loci (AFLP markers) for adaptive genetic divergence are those associated with sunlight intensity, water level, and pH, a finding consistent with the climatic differentiation and local environmental stresses playing a decisive role in the contemporaneous charophyte diversity.

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MORPHOTAXONOMIC AND MOLECULAR STUDIES OF CYANOBACTERIA OF DELTAIC REGION OF SOUTH EAST ASIA AND ASSESSMENT OF FATTY ACID PROFILE

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Along the south-east coast of Asia, Sundarbans is the largest single block of tidal halophytic mangrove forest in the world. It is a UNESCO World Heritage Site, covering parts of Bangladesh and the Indian state of West Bengal, with an area of 10,000 sq km. The Indian site lies between 21°31' to 22°53' N and 88°37' to 89°09' E. The algal communities of this coast are composed of several members forming a thick mat on the forest floor, exposed to regular tidal cycle showing a prominent salinity gradient. The area was surveyed and a detailed study of the cyanobacterial flora with proper identification was conducted. This study revealed that 30 percent of the algal flora was represented by the cyanobacterial members. *Lyngbya* and *Phormidium* were the dominant cyanobacterial genera followed by *Spirulina* and *Nostoc* along with other genera like *Synechocystis*, *Microcoleus*, etc.

Many species of *Lyngbya* and *Phormidium* were found in different salinity gradients of the habitat water. The growth performances and total lipid content of isolated genera were studied in laboratory condition. GCMS analyses of fatty acid compositions were analyzed. Molecular characterizations of dominant cyanobacterial flora were also studied in the present work.

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IMPORTANCE OF DRY PHOTOTROPHIC BIOFILM AS A REFUGE DURING A DROUGHT EPISODE – A MESOCOSM STUDY

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Non-permanent streams are widely distributed in the world but received only recently attention, in the context of global warming. To understand the impact of a drought episode on benthic phototrophic communities, we have conducted an experiment in an artificial channel with biofilms growing on artificial substrates. We have studied four periods of dewatering (1, 2, 4 and 8 weeks) and a chronic dewatering. For each case, we have simulated rewetting in individual aquariums during 13 days, to avoid exogenous inputs. Measures of PAM fluorescence, chlorophyll *a* and pheophytin and bacterial functional diversity have been done. Our results show that diatoms are more competitive during rewetting than chlorophytes and cyanobacteria when the drought period increases. Chlorophytes were still detectable during the dry phase for the chronic dewatering treatment but diatoms dominated the biofilm after rewetting. Cyanobacteria, initially dominant, were rapidly impacted by drought. After rewetting, the proportion of live and dead algae was relatively stable and equivalent. Though the recovery was rather low, this experiment emphasized the importance of dry biofilm as a source for re-growth after a drought episode.

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INVASION OF THE FRESHWATER DIATOM *DIDYMOSPHEA GEMINATA* IN SOUTHERN CHILE

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In Chile there is a new threat attacking rivers in Patagonia. The diatom *Didymosphenia geminata* or Didymo, is an exotic and highly invasive microalgae that has proliferated in Southern Chile since its first record on 2010. It forms nuisance blooms in low nutrient streams and its effects on native ecosystems, macroinvertebrates assemblages, food webs and fishes have not been documented. Its distributional range in Chile is spreading. On 2010-2011, the microalgae was detected in the Aysén (45.3 °S) and Los Lagos Region (43.2 °S), and in 2012 it was found further north at Los Ríos Region (39.5°S). The aim of this study was to detect new focuses of Didymo in two northern regions (38°S), in order to inform the authorities and apply measures intended to stop the spread of this invasion. Didymo was recorded in 4 out of 9 of the stations studied (present over 300 km), showing pH values over 7 and nutrient levels below 0.01mg/L. Due to these findings, an awareness workshop was performed and biosafety measures such as disinfection and other were implemented by the environmental authority.

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ALGAE AND THE BIOGEOCHEMISTRY OF CARBON

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Algae are major players in the global carbon cycle. They are responsible for approximately half the >110 Pg of inorganic C assimilated into organic matter every year through biological processes. Carbon fixation by algae through oxygenic photolithotrophy far surpasses the extent of carbon assimilation by other biological processes such as anoxygenic photolithotrophy and chemolithotrophy, though the former was quantitatively more important in the past than it currently is. In addition to the biosynthesis of organic matter, some algae are also responsible for the biosynthesis of calcium carbonate deposits, which contribute significantly to the drawdown of inorganic carbon from the atmosphere and have played a major role in the biogeochemistry of carbon on the planet. In this talk I will provide an overview of these processes as well as attempting to make predictions about how

they might be affected in the future though global change, including ocean acidification.

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IMPLICATIONS OF GLOBAL CLIMATE CHANGE FOR MARINE PHYTOPLANKTON

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Global increases in atmospheric CO₂ and temperature are associated with changes in ocean chemistry and circulation which in turn lead to altering light and nutrient regimes. Thus elevated temperatures will enhance stratification of the water column, leading to enhanced nutrient limitation and increased exposure to both photosynthetically active radiation and UV. Increases in dissolved CO₂ and direct effects of temperature rises will also have direct, species specific, impacts on processes such as photosynthesis, nutrient uptake and calcification. These changes are likely to impact upon phytoplankton populations by altering primary productivity, growth rate, cell size distributions and species composition and elemental stoichiometry. These in turn will have flow-on effects to food-webs, drawdown of biomass into the deep ocean (the biological C pump) and biogeochemical cycling. This paper will review the evidence for such changes using evidence from the literature on both natural populations and laboratory cultures.

23

IT'S THE SMALL THINGS (CYANOBACTERIA) THAT COUNT

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In most ecosystems, ecologists define the “critical zone” as the region where the majority of plant roots are concentrated. Thus, for instance, the critical zone in forested ecosystems is considered as the top several meters of soil. I propose a new way of thinking: that the critical zone is not necessarily where plant roots are found but is instead where the surface poikilohydric organisms, especially cyanobacteria, are found: that is, just above, on, or within the top few millimeters of rock, soil, or plant surfaces. This

extremely thin veneer of life is critical in almost every ecosystem process in many ecosystems, including weathering, C and N cycles, dust capture, bio-availability of P and metals, decomposition, and soil stability. In addition, as these communities cover most soil and rock surfaces, they mediate almost all inputs (water, gases, and nutrients) and outputs (gases) to underlying strata. They also facilitate delivery of C and nutrients from the soil interspace to plants. The direct connection between lithic communities and soil surface cyanobacterially-dominated communities and the connection between cyanobacteria living in the nutrient-rich soil surface zone and nearby vascular plants may be a critical process unique to dryland ecosystems.

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EFFECT OF PAST AND FUTURE CO₂ EMISSION SCENARIOS AND EUTROPHICATION ON THE COMMON CORAL REEF ALGA *CHNOOSPOR* *IMPLEXA*

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Macroalgae are associated with the decline of coral reef ecosystems. A shift in dominance from coral to algal dominated reefs has been associated with many factors inclusive of ocean eutrophication, acidification and warming. The actual response of macroalgae to a combination of these factors has, however, not been investigated. This study aimed to assess the effect of past and future CO₂ emission scenarios (applied as temperature and pCO₂ offsets to present day conditions) in isolation and in conjunction with nutrient enrichment on *Chnoospora implexa*. The experiment was conducted twice, once in winter and once in spring, to account for possible temporal effects. The results suggested that the timing of the experiment had a strong effect on the response variables tested. An interactive effect between time and emission scenario was observed for the biomass data, suggesting that the greatest growth occurred in spring under pre-industrial CO₂ and temperature conditions. Nutrient enrichment led to luxury nutrient uptake, had only a mild effect on dark respiration and did not affect net maximum productivity.

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SHEDDING LIGHT ON EUGLENOID EVOLUTION AND SYSTEMATICS THROUGH THE CHLOROPLAST GENOMES OF *EUGLENA VIRIDIS* AND *EUGLENA PROXIMA*

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The chloroplast genomes of *Euglena viridis* and *Euglena proxima* were sequenced and analyzed. Intrageneric chloroplast evolution was explored with the chloroplast genome of *E. viridis*, and results revealed that while the chloroplast genome of *E. viridis* closely resembled that of *Euglena gracilis*, it did show significant differences: The chloroplast genome of *E. viridis* was more compact, had a gene cluster that was reversed in order and strand orientation, had a region that was comprised almost entirely of open reading frames, and had substantially fewer introns than *E. gracilis*. The chloroplast genome of *E. proxima* was sequenced in order to clarify its relationship to the rest of the photosynthetic euglenoids. Seventy-eight chloroplast-encoded genes from *E. proxima* were used in phylogenomic analyses with those from six photosynthetic euglenoids and three prasinophytes. The results of these analyses were consistent with previous phylogenetic analyses, and supported the position of *E. proxima* as sister to all of the Euglenaceae. These data further indicate that *E. proxima* does not belong in the genus *Euglena*, and that a new genus must be erected for this taxon.

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COMPARATIVE TRANSCRIPTOMICS OF GREEN ALGAE: RELATING SPECIES INTERACTIONS TO DIFFERENTIAL GENE EXPRESSION

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Evolution should lead to genetic divergence and niche differentiation among species, so that phylogenetic relationships should predict the strength of competition for resources among species. As part of a highly collaborative NSF-funded Dimensions of Biodiversity

Project, we are investigating niche partitioning in assemblages of green algae. We reared pairs of green algal species of different degrees of phylogenetic relatedness and estimated strength of competition in these cultures using the Lotka-Volterra model. Our aim is to relate competition strength and phylogenetic distance in these experimental cultures to profiles of gene expression. If competition for resources is high among species we expect little divergence in gene expression among these species. By contrast, if competition is low we expect to observe appreciable differences in gene expression that may be related to differential resource exploitation. In order to investigate genome-wide levels of gene expression we are using short, 100bp paired-end sequences, as generated by Illumina sequencing technology. By generating unprecedented amounts of sequence data, Illumina, and other second generation sequencing technologies, have the potential to facilitate the analysis of genome-wide gene transcription, even for organisms that lack well-characterized reference genomes. We sequenced the mRNA of a set of eight algal species grown in monoculture and biculture in all possible pairwise combinations. No reference genome or transcriptome exists for any of our study species, so that our analysis work-flow includes assembling the transcriptomes of each species *de novo* from the sequence data we generated. Using these newly assembled transcriptomes as a reference, we are able to estimate gene expression in our competition experiments, and relate levels of gene expression to metabolic pathways and/or other functional categories of genes.

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A TAXONOMIC STUDY ON *CALOTHRIX* – GROUP (CYANOBACTERIA) BASED ON MORPHOLOGY AND ANALYSIS OF THE 16S RRNA GENE FRAGMENT

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The genus *Calothrix* Agardh ex Bornet et Flahault 1886 is characterized in the botanical literature on the basis of morphological features such as tapering of

their trichomes, presence of basal heterocysts or development of multicellular hairs at the end of apical part. However, some of these characteristics, for example the ability of the hair formation, are common in other genera and proved to be dependent on environmental and cultural conditions. Thus, reliable identification of a culture based solely on morphological features is quite difficult. Because of this, the taxonomic status of the genus *Calothrix* has been discussed widely. Moreover, phylogenetic analysis of 16S rRNA gene sequences, and relationships between strains of the genus *Calothrix* deduced from DNA–DNA hybridization results have demonstrated a high genetic diversity in this genus. These results point out that *Calothrix* is not a coherent group and needs a taxonomic revision in depth, where species are redefined on the basis of the morphological and genetic properties. This only can be accomplished with the application of modern and combined criteria, following the called polyphasic approach.

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THE *CYANOPHORA* GENOME: ANALYSIS OF GENE AND SMALL RNA CONTENT IN A PIVOTAL ALGAL LINEAGE

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The primary endosymbiotic origin of the plastid in eukaryotes more than 1 billion years ago led to the evolution of algae and plants. We generated a draft genome and transcriptome data from a member of the anciently diverged algal lineage *Cyanophora paradoxa*. Our work provides evidence for a single origin of the primary plastid in the eukaryote supergroup Plantae (also known as Archaeplastida). *C. paradoxa* retains ancestral features of starch biosynthesis, fermentation, and plastid protein translocation common to plants and algae, but lacks typical eukaryotic light harvesting complex proteins. We also analyzed the small RNA (sRNA) pool in *C. paradoxa*. RNAi (RNA interference) relies on the production of sRNAs from double-stranded RNA and comprises a major pathway in eukaryotes to restrict the propagation of selfish genetic elements. Amplification of the initial RNAi signal by generation of multiple secondary sRNAs from a targeted mRNA is catalyzed

by RNA-dependent RNA polymerases (RdRPs). This phenomenon is known as transitivity and is particularly important in plants to limit the spread of viruses. A genome-wide approach with *C. paradoxa* shows that sRNAs in this species are preferentially associated with mRNAs, including a large number of transcripts that encode proteins arising from different functional categories. This pattern of exonic sRNAs appears to be a general trend that affects a large fraction of mRNAs in the cell. In several cases we observe that sRNAs have a bias for a specific strand of the mRNA, including many instances of antisense predominance. The genome of *C. paradoxa* encodes four sequences that are homologous to RdRPs in *Arabidopsis thaliana*. We discuss the possibility that exonic sRNAs in the glaucophyte may be secondarily derived from mRNAs by the action of RdRPs. If this hypothesis is confirmed, then transitivity may have had an ancient origin in Plantae.

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BIOFUEL CROP IMPROVEMENT USING EXPERIMENTAL EVOLUTION

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Improving the growth rate and providing crop protection are key targets of strain improvement with algal biofuel candidates. Here we used a strategy of experimental evolution with the model green alga *Chlamydomonas reinhardtii* strain CC-503 to assess the potential of strain improvement using natural selection with lab cultures. We evolved laboratory populations of *C. reinhardtii* for over 1,200 generations in TAP medium under continuous light in one experiment and under continuous light and 200 mM NaCl in another. Assessment of growth rates showed fitness gains during the experiment for both cell populations. To elucidate the basis of salt tolerance (as a proxy for crop protection) in the alga, we re-sequenced the genome and generated mRNA-seq data from the progenitor and evolved light and light + salt populations. These analyses demonstrate a significant restructuring of the *C. reinhardtii* transcriptome due to adaptation to high growth rates (to outgrow con-specific competitors) and to survive salt stress. These fast-growing and salt resistant

populations can be easily manipulated to produce lipids by depletion of nitrogen in the medium. We will present the results of this work and discuss the genome-wide impacts of experimental evolution on algal biology and its potential for strain improvement.

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PHYLOGENY AND TAXONOMY OF GELIDIUM DIVARICATUM (GELIDIACEAE, RHODOPHYTA): A CANDIDATE NEW GENUS OF MARINE ALGAE FROM EAST ASIA

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We conducted phylogenetic analyses of plastid *rbcL* and mitochondrial *cox1* sequences and detailed morphological observations on specimens of *Gelidium divaricatum* collected in Korea, China, Japan, as well as Hong Kong, the type locality of the species. It is one of the most common mat-forming species and commonly found on rocks in the upper intertidal zone in East Asia. In all analyses of individual and combined datasets, *Gelidium* was segregated into four different clades; i) the main *Gelidium* group, ii) *G. divaricatum* group, iii) *Capreolia/Gelidium caulacanthum* group, and iv) Chilean *Gelidium* group. The *Gelidium divaricatum* clade, is distantly related to the main group of *Gelidium*, and also clearly separated from the *Capreolia/Gelidium caulacanthum* group and the Chilean *Gelidium* spp. group. These three groups are also geographically separated; *G. divaricatum* from East Asia, *Capreolia/Gelidium caulacanthum* group from Australasia, and two *Gelidium* spp. from Chile. Here we describe a new genus and species based on specimens, previously referred to as *Gelidium divaricatum* in Korea, China, and Japan, and also propose the new combination for the taxon based on *Gelidium divaricatum* G. Martens.

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COMPARATIVE ANALYSES OF EFFECTS OF THE ULTRAVIOLET B RADIATION ON CELL ORGANIZATION AND METABOLISM IN THE AGAROPHYTE AND CARRAGENOPHYTE ALGAE

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The *in vitro* effects of UVBR were investigated in apical segments of *Chondracanthus teedei*, *Hypnea musciformis*, *Gracilaria domingensis* and *Gelidium floridanum*. The plants were cultivated and exposed to photosynthetically active radiation (PAR) at 80 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ and PAR + UVBR at 1.6 W m^{-2} at 3 h per day for 7 and 21 days. UVBR caused changes in the ultrastructure of cortical cells, which included increased thickness of the cell wall and destruction of chloroplast organization. The algae cultivated under PAR + UVBR showed a decrease in growth rates. Furthermore, compared with control algae, phycobiliprotein contents (phycoerythrin, phycocyanin, and allophycocyanin) were observed to decrease after PAR + UVBR. *C. teedei* and *G. domingensis* showed a decrease in chlorophyll *a* levels. However, chlorophyll *a* levels in *H. musciformis* and *Ge. floridanum* were not significantly different after exposure to PAR + UVBR. Overall, these results lead to the conclusion that both ultrastructural damage and observable changes in metabolism occurred in four species after only 3 h of daily UVB exposure over a 7 and 21-d experimental period.

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TARA OCEANS: COMPREHENSIVE BIOGEOGRAPHIC INSIGHTS INTO THE COMPLEXITY OF MARINE DIATOM COMMUNITIES

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With biology becoming quantitative, systems level studies can now be performed at spatial scales ranging from molecules to ecosystems. Biological data generated consistently across scales can be integrated with physico-chemical contextual data for a truly holistic approach. While the marine planktonic ecosystems that diatoms inhabit comprise the base of the ocean food web, and are crucial in the regulation of Earth's biogeochemical cycles and climate, their organization, evolution and dynamics remain poorly

understood. The Tara Oceans expedition was launched in September 2009 for a 3-year study of the global ocean ecosystem aboard the schooner *Tara*. A unique sampling program encompassing optical and genomic methods to describe viruses, bacteria, archaea, protists and metazoans in their physico-chemical environment has been implemented. The project aims to generate systematic, open access datasets usable for probing the morphological and molecular makeup, diversity, evolution, ecology and global impacts of plankton on the Earth system, as well as to explore and exploit their biotechnological potential. Using the unique Tara Oceans dataset we are exploring diatom abundance and biodiversity in the world's oceans.

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RELATIVE CONTRIBUTION OF ENVIRONMENTAL AND SPATIAL PROCESSES IN STRUCTURING STREAM MACROALGAL COMMUNITIES

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Several studies have suggested that the structure of stream macroalgal communities is shaped by environmental variables at a local scale. However, recently it has been shown that the importance of local environmental factors may be being overestimated, suggesting that regional processes could also be relevant. In this sense, the interdependency of environment and space, based on the theory of the metacommunity, must be considered in determining the community structure of stream macroalgae. Thus, considering this perspective, we examined the relative importance of environmental characteristics (water temperature, conductance, turbidity, dissolved oxygen, pH, velocity, depth, total nitrogen, and total phosphorous) and spatial arrangement (distance) of 105 sampling sites from southern Brazil on taxonomic composition of stream macroalgal communities. Partial redundancy analysis indicated that taxonomic composition was significantly explained by both space and environment for entire community, and for Chlorophyta and Cyanobacteria, but with higher contribution of environmental factors. For Rhodophyta only space was significant. The divergence in the

relative contribution of spatial and environmental predictors among the algal divisions can be ascribed to the differences in dispersal ability and ecological tolerance of each algal group.

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LIGHT AVAILABILITY AFFECTS THE OCEAN ACIDIFICATION RESPONSE IN COCCOLITHOPHORES

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Ocean acidification (OA) is predicted to profoundly affect biological processes that utilize inorganic carbon, such as calcification and photosynthesis. Much of the OA research upon phytoplankton has focused on the globally distributed calcifying coccolithophore species *Emiliania huxleyi*, but the data between studies has been highly inconsistent, and often contradictory. Additional environmental factors, such as nutrient and light availability, that can influence the OA response, have not been fully considered. Here we examine for the first time the influence of light dose (daylength) on coccolithophore's response to OA. Under a 14:10h L:D cycle, the growth rates of *E. huxleyi* strain NZEH decreased with elevated CO₂ (from 1.07 d⁻¹ at 385 ppm to 0.75 d⁻¹ at 1000 ppm), but calcification remained unchanged (coccolith thickness). However, this response was reversed under continuous light, where growth rate was unaffected by CO₂ concentration (0.93-0.94 d⁻¹ for both CO₂ treatments), but calcification was reduced at 1000 ppm. We examined whether this model response applied to other *E. huxleyi* strains (isolated from different geographical locations and age), as well as two other species of coccolithophore, *Coccolithus pelagicus* and *Gephyrocapsa oceanica*. All three of the *E. huxleyi* strains tested demonstrated a similar change in OA response with L:D cycle as the NZEH strain. Both *G. oceanica* and *C. pelagicus* showed depressed growth rates at 1000 ppm CO₂ regardless of light regime, though calcification in *G. oceanica* decreased with CO₂ only under continuous light. We are currently testing this aim more widely upon natural phytoplankton communities (often containing significant contributions from coccolithophores) in polar waters, i.e. areas of the world that experience

natural extreme variation in daylength but are predicted to be vulnerable to OA and climate change; these results will be discussed in the context of observations from the laboratory grown cultures.

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WHAT WOULD THE WORLD BE LIKE WITHOUT *CORALLINA*: DIVERSITY, SKELETAL MINERALOGY AND MICROBIOMES OF THIS CALCIFIED GENUS IN A TIME OF UNPRECEDENTED CHANGE

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What would the world be like without coralline algae? In shallow benthic ecosystems we would lose i) genetic diversity, ii) extensive 3D habitats that greatly increase benthic diversity by providing hard substrata for other organisms, iii) sequestration of carbon, iv) a skeleton whose mineralogy provides a record of sea surface temperature, and v) a microbiome whose role has yet to be ascertained. To understand these organisms we are undertaking an integrated study of the calcified genus *Corallina*, a cosmopolitan rhodophyte. Molecular phylogenetic studies have revealed considerably more diversity than previously described, with our recent global analyses identifying up to 15 undescribed species. X-ray diffraction analysis of *Corallina* skeletal mineralogy across the recent-past (1850 – 2005) and present-day, revealed temperature-dependent seasonal and latitudinal patterns in skeletal magnesium incorporation, highlighting *Corallina* vulnerability to climate change and ocean acidification. We will also review our work using next generation sequencing to describe the *Corallina* microbiome. Our results will be discussed in relation to patterns of species distribution, increased sea surface temperatures and ocean acidification.

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PHYSIOLOGICAL RESPONSES OF *MACROCYSTIS PYRIFERA* TO TEMPERATURE AND CO₂

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Anthropogenic climate change is a major threat to global biodiversity and ecosystem health. In the last century, average global atmospheric temperatures have risen roughly 1°C, with sea surface temperatures rising concurrently. This rise has been attributed to increases in global atmospheric CO₂, the majority of which is driven by human activities. In addition to changing the world's temperature, CO₂ emissions are altering the carbon chemistry of the ocean, shifting the distribution of carbon between species and resulting in larger amounts of diffuse CO₂ and a decrease in pH. All of these factors have the potential to drastically affect the health and stability of coastal ecosystems. While many studies have examined the effects of climate change on marine algae, much of the focus has been on phytoplankton and coralline macroalgae, and we know comparatively little of how climate change will affect non-calcifying species. For this study we chose the giant kelp *Macrocystis pyrifera*. *Macrocystis* is the primary canopy-forming kelp along the southern coast of California, providing food and habitat for a large variety of species. We cultured apical tips of *Macrocystis* for one month in laboratory mesocosms under four conditions; ambient (12°C and 500 µatm), elevated temperature (15°C and 500 µatm), elevated CO₂ (12°C and 1500 µatm), and elevated temperature and CO₂ (15°C and 1500 µatm). Growth rates were measured weekly, while photosynthesis and chemical composition was measured at the end of the experiment. Kelps cultured under elevated temperature showed significant reductions in growth and photosynthesis, while elevated CO₂ had no significant effect on these parameters. Interestingly, kelps cultured under both factors together showed greatly increased rates of photosynthesis and growth. These results suggest that *Macrocystis* may benefit physiologically from climate change, and that examining individual factors in isolation may present an inaccurate and incomplete picture.

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UNDERSTANDING THE MECHANISMS OF CELL AND POPULATION RESPONSES OF COCCOLITHOPHORES TO CHANGING OCEAN CHEMISTRY

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The calcifying coccolithophores have been proposed as a potentially vulnerable group in the face of increasing surface ocean CO₂ levels. A full understanding of the likely responses of this group requires better mechanistic information on pH- and CO₂-sensitive processes that underlie cell function at molecular, cellular and population levels. New findings on the mechanisms of pH homeostasis at a molecular and cellular level in both diatoms and coccolithophores are shaping our understanding of how these important groups may respond or acclimate to changing ocean pH. Critical parameters including intracellular pH homeostasis and cell surface pH will be considered. These studies are being carried out in parallel with genetic studies of natural oceanic populations to assess the natural genetic and physiological diversity that will underlie adaptation of populations in the long term.

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CYANOBACTERIAL SOIL CRUSTS OF TROPICAL SAVANNAS – THEIR DIVERSITY AND ECOPHYSIOLOGICAL PROPERTIES

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Biological soil crusts (BSC) are the oldest ecosystems on the land surface of the Earth. Initially they were formed by cyanobacteria and still nowadays, most BSCs are initiated and often even dominated by cyanobacteria. Spreading on about 30 % of the Earth's land surface, cyanobacterial dominated BSCs especially occur in the savanna biome of the refreezing continents. Cyanobacterial BSCs have been reported from the savannas of Australia, South America (Venezuela), Africa (Kenya, Tanzania, Namibia, Niger, Nigeria, South Africa), and Asia (India, Sri Lanka). Initiated by filamentous cyanobacteria of the genera *Coleofasciculus*, *Microcoleus*, *Nostoc*, *Schizothrix*, *Symplocastrum*, and *Trichocoleus*, these crusts are forming a favorable habitat for other cyanobacteria and green algae in later successional stages. Among the ecosystem services of those BSCs

are soil stabilization and nutrient input (C, N). Depending on the developmental stage of the BSCs (initial crust, well developed crust and old crust), they contribute to the CO₂ fixation of the whole savanna biome. In this talk I will give an overview on the distribution and types of BSCs in savannas worldwide will be given as well as information about their cyanobacterial species composition and the photosynthetic properties of the resulting crust types.

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ECOPHYSIOLOGICAL RESPONSES OF ANNUAL POPULATIONS OF *MACROCYSTIS PYRIFERA* UNDER WINTER LIGHT CONDITIONS

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To understand the success of recruitment, survival and early growth of annual populations of *Macrocystis pyrifera*, field and experimental studies were carried out in southern Chile. This study provides evidence about the environmental factors driving the recovery of the sporophytic populations in late winter and early spring by monitoring two independent kelp populations, and also by using 400-L tubular photobioreactors with semi-controlled environmental factors. The sporophytes established on late winter/early spring are able, under high nitrogen availability, to increase their chlorophyll content significantly, allowing the individuals to reduce their light saturation point and therefore having a higher productivity under the low light conditions existing in late winter and early spring. These results help to explain how annual giant kelp populations can successfully couple successive sporophytic generations and also emphasize the highly plastic physiology of giant kelp enabling this species to colonize diverse habitats along its large distributional range.

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TWO NEW SPECIES OF *NEOSIPHONIA* (CERAMIALES, RHODOPHYTA) FROM BALI, INDONESIA

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The genus *Neosiphonia*, segregated from *Polysiphonia* by Kim & Lee in 1999, currently has 32 recognized species worldwide. Herein, we describe two unidentified samples collected from Bali, Indonesia, as new species based on morphological and molecular data. *Neosiphonia* sp-1 is recognized by having rhizoids cutting off from the proximal end of pericentral cells, 5 pericentral cells, ecorticated axes through thallus, trichoblasts and scar cells absent, straight arrangement of tetrasporangia, and spermatangia arisen from a basal cell. *Neosiphonia* sp-2 is characterized by having rhizoids cutting off from the proximal end of pericentral cells, 4 pericentral cells, ecorticated axes through thallus, abundant trichoblasts near the apex, prominent scar cells, exceedingly prominent apical cell, and one or two tetrasporangia per branch. Although, some species have mixed features, the rhizoids connection is a strong indicator used to delimit *Polysiphonia sensu lato* species in the absence of female stage. Phylogenetic analyses of *rbcL* indicated that our *Neosiphonia* sp-1 and *Neosiphonia* sp-2 are placed in genus *Neosiphonia* and are also distinguished from other *Neosiphonia* species.

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ITS2 - A UNIVERSAL MOLECULAR TOOL FOR THE IDENTIFICATION OF SPECIES?

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Species are fundamental units in biology. In protists, a commonly used method of species delineation is based on the identification of Compensatory Base Changes (CBCs) in conserved regions of the ITS2 molecule (the spacer between the 5.8S rDNA and the 28S rDNA). Recent studies on ITS2 evolution in several lineages of green algae (Ulvales, Chaetophorales, Chaetopeltidales, Oedogoniales and Sphaeropleales), however, revealed that CBCs are not diagnostic at the species level, even genera, families and orders could lack CBCs in such regions. Moreover, we found that criteria/landmarks used, e.g., for automatic prediction of the ITS2 secondary structure, mostly fail to identify the starting points of ITS2 Helices. This often invalidates robust identification of CBCs and thus of

species based on spurious CBCs. Here we present (1) the first consensus secondary structure model of the ITS2 molecule that can be applied to two classes of green algae (Chlorophyceae, Ulvophyceae-Ulvales), and (2) new landmarks (the spacer regions separating the ITS2 Helices) for robust prediction of the ITS2 secondary structures in general.

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NEW INSIGHTS IN THE DIVERSITY OF THE GENUS *LOBOPHORA* (DICTYOTALES, PHAEOPHYCEAE) BASED ON MOLECULAR AND MORPHOLOGICAL EVIDENCE

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Lobophora J. Agardh is one of the most abundant crustose, prostrate or erect genera growing in coral reefs and in other tropical and subtropical environments. Although AlgaeBase currently lists *Lobophora* as comprising ten species, species diversity in this genus has proved to be underestimated much more than was previously thought. Chloroplast-encoded *rbcL* and *cox3* sequence analyses conducted on western Atlantic and Red Sea specimens indicate newly found diversity representing four distinct species, with one unreported species each for Caribbean Colombia, Caribbean Panama, the NW Gulf of Mexico, and Egypt. These taxa in all likelihood correspond to new species. In addition, we propose range extensions for previously unnamed *Lobophora* spp. reported in Sun *et al.* 2012. Three recently collected species from the Red Sea are conspecific with recently characterized taxa from Japan, Palau and Malaysia, and one species from the NW Gulf of Mexico is conspecific with a sample from Curaçao in the Lesser Antilles. The morphological evidence for describing the new species of *Lobophora* will be discussed in light of the molecular-based results.

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A DNA BARCODE APPROACH OF THE *LAURENCIA* COMPLEX (CERAMIALES, RHODOPHYTA) IN THE TROPICAL AND SUBTROPICAL ATLANTIC OCEAN

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The diversity of the *Laurencia* complex is being assessed in tropical and subtropical Atlantic by an international cooperation project involving Brazil, Mexico, Spain (Canary Islands), Portugal (Azores and Madeira) and USA (Florida) on the base of molecular data allied to a detailed morphological study of species. The diversity of the complex was analyzed for the first time for the Atlantic Ocean, including specimens from all five localities, using the plastid 23S rRNA gene (UPA) which has been investigated as potential DNA Barcode marker for photosynthetic eukaryotes. The mitochondrial cytochrome c oxidase I gene (COI-5P) was also used as DNA barcode for the same set of species, and the *rbcL* gene was used for phylogenetic inferences. The range of genetic variation was compared for the three markers. The UPA proved to be more conserved; however, the same genetic groups were resolved with each of the three markers confirming the six genera currently established for the complex: *Chondrophyucus*, *Laurencia*, *Laurenciella*, *Palisada*, *Osmundea* and *Yuzurua*.

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CHARACTERIZATION OF *BATRACHOSPERMUM GELATINOSUM* (L.) DE CANDOLLE AND *B. ARCUATUM* KYLIN (BATRACHOSPERMALES, RHODOPHYTA) FROM THE IBERIAN PENINSULA

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Freshwater red algae diversity in the Iberian Peninsula (Spain and Portugal) has been poorly studied. The

purpose of this study is to approach the morphological and genetic variation of two most common members of the Batrachospermales in the study area, to better understand their biogeographic and phylogenetic relationships in a more global context. We compared genetically six populations each of *B. gelatinosum* and *B. arcuatum* from eight different river basins, using three molecular markers to evaluate genetic diversity: RuBisCo large subunit (*rbcL*) (fully sequenced at the moment), cytochrome oxidase 2-3 spacer (*cox2-3*) and the barcode region of cytochrome oxidase I (*cox1*) (preliminary data available). For the morphological comparison nine additional populations were included in the analysis. A wide morphological variation was observed for most vegetative and reproductive characters. *rbcL* sequences showed a relatively low genetic divergence: 98.8-100% for *B. gelatinosum* and 99.9-100% for *B. arcuatum*. We found no correlation between genetic diversity and morphological variation among the populations of both species. Some taxonomic characters are reevaluated aiming at a more reliable characterization of these species.

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GROWTH PERFORMANCE OF TWO OLEAGINOUS MICROALGAE *NANNOCHLOROPSIS OCEANICA* IMET1 AND *N. GADITANA* CCMP526 UNDER DIFFERENT CULTIVATION CONDITIONS

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Recently, the genome sequences of *Nannochloropsis oceanica* IMET1 and *N. gaditana* CCMP526 have indicated that these two algal strains have advanced lipid metabolism pathways and are promising microalgae for biofuel development. It would be interesting to know how these two strains respond to the changes of environments. Here we compared the growth performance of these two strains under various levels of CO₂ concentration, temperature, and light intensity. All the experiments were performed in two different culture media (ASW and BG11) with six replicates. The optical density (OD₆₀₀) was measured using a microplate reader every two days. Both strains (IMET1 and 526) had the highest growth rate with 2% CO₂, and their growth rates decreased with increasing

CO₂ levels. At the levels of 10, 15 and 20% CO₂, IMET1 outperformed 526, suggesting that IMET1 can grow in higher CO₂ concentrations than strain 526. At three different temperatures (10, 23 and 30°C), IMET1 grew the best at 30°C, and exhibited higher growth rates than 526 in general. Under three light intensities (13, 53, 82, 132 μmol/m²/s), IMET1 showed the higher growth rates than 526. The growth of both strains was photo-inhibited at the high light level. Interestingly, both strains preferred BG11 medium over ASW medium in most cases. Our experiments suggest that *N. oceanica* IMET1 appears to have wider environmental tolerance capability compared to *N. gaditana* CCMP526, and could be more suitable for the outdoor cultivation system where environmental conditions are rigorous. The lipid content, fatty acid composition and metabolic profile were investigating via different cultivation conditions. And we got a high yield of EPA with a two-stage cultivation. We will present the results in the meeting.

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PRESENT DAY COLLECTIONS OF FRESHWATER RED ALGAE (BATRACHOSPERMALES, RHODOPHYTA) FROM HISTORICALLY IMPORTANT SITES IN FRANCE

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An interest in freshwater red algae began in France over 200 years ago. Bory de St. Vincent described new species of *Batrachospermum* and *Lemanea* from Thore's collections made near Dax in Gascony and from his own collections near Rennes in Brittany. Additionally, Sirodot and Gallée collected extensively around Rennes. These specimens formed the basis of Sirodot's seminal work on *Batrachospermum* in which numerous new species were described. Since Sirodot's work, there has been limited research on these organisms in France. The purpose of the present research was to revisit these historically important streams. In March 2008, four streams near Rennes were visited yielding five freshwater red algal samples identified as two *Batrachospermum* and one *Lemanea* species. In October 2011, Rennes was revisited and 45 streams sampled; one *Paralemanea* and three *Batrachospermum* species were collected. In addition,

fifteen streams near Dax were visited providing 17 *Batrachospermum* samples identified as five distinct species. Although there have been changes in land use and habitat, many of the taxa first described by French naturalists are still present in the flora today.

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MORPHOMETRIC, ULTRASTRUCTURE AND MOLECULAR STUDY OF THE GRACILARIOIDS FROM THE EAST COAST OF THE GULF OF THAILAND

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Studies on the Thai seaweeds are mainly based on morphology, which information of fine structural and molecular aspects is scarce. This study *Gracilaria* sp. *G. firma*, *G. salicornia* and *Hydropuntia changii* collected from the east coast of the Gulf of Thailand were analyzed using cytology and RAPD techniques associated with morphology to distinguish population of the seaweed. The results showed the decklamelle and outer wall in all specimens are continuous when viewed with the light microscope. The transmission electron micrographs (TEM) distinguished the structure of each species with floridean starch granules distributed around the nuclear content. *G. salicornia* showed clearly chloroplasts with parallel thylakoids and pit plug. Anomalous chloroplasts with thylakoid disorganization were found in cortical and medullar cells of *H. changii* and *Gracilaria* sp. The discriminant analysis (n=30) showed *H. changii* specimens close to *Gracilaria* sp. whereas *G. firma* and *G. salicornia* were distinguished from *H. changii* and *Gracilaria* sp. The specimens were clustered by unweighted pair group method using arithmetic averages (UPGMA). The cluster analysis showed that *Gracilaria* sp. had a relation closer to *G. salicornia* than *H. changii* and *G. firma*. The results obtained were correspondent to the RAPD analysis.

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DEVELOPMENT OF A MUTANT SPIROGYRA VARIANS STRAIN WITH HIGH STARCH CONTENT BY GAMMA IRRADIATION BREEDING METHOD

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Recently, numerous studies have focused on the development of high-efficiency plants for the production of biofuels. Algae such as *Chlorella*, *Dunaliella*, *Chlamydomonas*, *Scenedesmus*, and *Spirogyra* are known to contain large amounts (>50% of the dry weight) of starch. These algal starches can be used as biomass for ethanol production after hydrolysis. Research suggests that *Spirogyra* has important potential as a source of biofuel due to its high biomass content (>43% of the dry weight) and its ability to grow rapidly under diverse growth conditions, including the winter season in fallow land where fresh water is available. But, for the economical production of biomass, more productive strains should be developed. However, the development of improved algae using molecular biological tools has been demonstrably less successful than similar efforts with bacteria and land plants, due in part to the difficulty in transforming algae. Radiation breeding has been used for the developing the superior bacterial strains and plants. Therefore, in this work, we report research efforts to increase starch content for biomass production with *Spirogyra varians* via gamma irradiation. The resulting mutant was evaluated with respect to starch content, growth potential, and pigment content. The change in proteome level of the mutant was also evaluated in comparison with the wild-type, using two-dimensional electrophoresis (2DE) protein profiling. The results are discussed in terms of the functional differences in identified proteins between the wild-type and mutant strains.

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PHYLOGENETIC RELATIONSHIP OF VARIOUS CERAMIUM SPECIES (CERAMIACEAE, RHODOPHYTA) FROM FLORIDA, USA

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To characterize the species of *Ceramium* in Florida and infer their phylogenetic relationships, *Ceramium* collections were made from 2002 to 2005. An rbcL

dataset was compiled from over 30 samples. Investigated species with complete or incomplete cortication include *C. nitens*, *C. corniculatum*, *C. brevizonatum* and some undescribed species from Florida. Each species is recognized by the number of periaxial cells, development of cortication, branching pattern, shape of the apex, and habit. Twelve clades were resolved in a variety of positions within the *rbcL*-based phylogenetic tree and from combined molecular and morphological analyses.

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MORPHOLOGY, PHYLOGENETIC RELATIONSHIPS AND DNA BARCODING OF THE BANGIALES (RHODOPHYTA) FROM KING GEORGE ISLAND, ANTARCTIC AND ITS ADJACENT WATERS

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Members of the Bangiales (Rhodophyta) are distributed worldwide from the tropics to the Antarctic and the Arctic. Three species of the Bangiales, *Bangia fuscopurpurea* (as *B. atropurpurea*), *Porphyra plocamiestris* and *Pyropia endiviifolia* (as *Porphyra endiviifolium*), have been reported in the Antarctic. Morphological and molecular data were investigated for the Bangiales from the Antarctic and its adjacent waters. Several tens of sequences were newly determined in this study for each of the SSU rDNA, plastid *rbcL* and mitochondrial *cox1* genes. Molecular data from about 200 taxa of the Bangiales worldwide including previously published sequences, indicated that the genera *Bangia*, *Dione*, *Pyropia*, *Porphyra*, *Wildemania* and other related genera be recognized in the Bangiales, as was found in a previous molecular study. *Bangia fuscopurpurea* from the Antarctic was different from *B. fuscopurpurea* from north Pacific (Korea and Japan) by 11-12 bp in *cox1* gene sequence. *Porphyra plocamiestris* growing on other macroalgae in the subtidal zone grouped into the genus *Wildemania* with other species having one or two cell layers. The olive green *Pyropia endiviifolia* allied to a clade with *P. cinnamomea* and *P. virididentata* from

New Zealand. The taxonomic issues and phylogenetic relationships of the Antarctic members of the Bangiales will be discussed.

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A PERSISTENT BLOOM OF ANADYOMENE J.V. LAMOUREUX (ANADYOMENACEAE, CHLOROPHYTA) IN BISCAYNE BAY, FLORIDA

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Green macroalgal blooms are becoming a common response to increasing nutrient availability in coastal waters and estuaries. This study describes the first occurrence of a persistent macro algal bloom of the genus *Anadyomene* Lamoroux (Cladophorales, Anadyomenaceae) in Biscayne Bay, Florida. A molecular analysis sequencing the variable C1D2 region of the large subunit (LSU) nrDNA verified the morphological-based identification. Results indicate that the bloom is composed of two species: *Anadyomene stellata*, previously known for Florida and Biscayne Bay, but is for the first time reported to reach blooming abundances. The second species, *A. linkiana*, a diminutive perforate species from the Bahamas, represents a new record for the United States, and an expansion of its known ecological range from deep to shallow waters. Large patches of *Anadyomene* were detected in 2005. Intensive surveys based on a stratified random design, to visually estimate the percent cover of submerged aquatic vegetation, were conducted in the bloom area once a year from 2010 to 2012. Results show that the *Anadyomene* bloom covered an area of approximately 80 km² of seagrass habitats. The *Anadyomene* spp. reached bloom densities in 2008, and has persisted through 2012. The spatial distribution of the bloom has been restricted to the central inshore section of the Bay, an area affected by canals and groundwater discharges. The persistent 75% cover reported for several sites has caused significant negative impacts to seagrass beds. This bloom occurring adjacent to metropolitan Miami, adds to the world trend of

increasing green macroalgal blooms occurring at enriched coastal waters.

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INSIGHTS TO ALGAL EVOLUTION FROM THE *CHONDRUS* GENOME

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Chondrus crispus (Irish moss) is a common red seaweed found on rocky shores of the Northern Atlantic. The 105-Mbp genome of this florideophyte has an unusual structure with gene-dense regions with short intergenetic distances surrounded by repeat-rich regions dominated by transposable elements. Our annotation of the genome predicts 9,606 genes with few and small introns, 88% of genes are without introns. Most gene families are relatively small and some families such as sulfatases, selenoproteins, the machinery for DNA methylation and many photoreceptors were not found. The gene and genome structures and gene content allowed us to propose an evolutionary scenario with gene loss and genome compaction, including loss of introns, in an ancestral red alga followed by a genome expansion through activity of transposable elements. The newly sequenced genomes of two other red algae with different evolutionary history, *Pyropia yezoensis* and *Galdieria sulphuraria*, permit us to test this hypothesis.

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PHYCOLOGICAL EDUCATIONAL ENDEAVORS: ASSESSING ALGAL KNOWLEDGE IN MUSEUMS, ZOOS, AQUARIUMS, AND HERBARIUMS

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Public knowledge of algae is often limited. For example, initial reactions to the word “algae” are frequently neutral to negative in tone. To gain perspective into how phycology is taught to public audiences (ranging from children to adults) the Phycological Society of America (PSA)’s Education Committee developed and deployed an online survey to assess how algae are curated, displayed, and interpreted in museums, zoos, aquariums and herbariums. These data will inform the PSA Education Committee on how to improve algal education across a wide spectrum of audiences and knowledge bases.

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DIVERSITY OF GRACILARIACEAE IN BRAZIL BASED ON MOLECULAR MARKERS

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Due to their ecological and economic importance, species of Gracilariaceae have been widely studied. However, taxonomy difficulties persist, especially in flattened tropical species. Here we present novel sequences of UPA and 5'cox1 for several species from Brazil and elsewhere maintained in culture in a germplasm bank. The rbcL gene was sequenced to enable comparison with other sequences in the databases. A total of 289 sequences were generated for 135 samples. Molecular data indicates 19 species of *Gracilaria* and two of *Gracilariopsis* on the Brazilian coast, of which three are new records for Brazil: *Gracilaria damaecornis*, *G. hayi* and *Gracilariopsis silvana*. Based on morphological and molecular data *Gracilaria tepocensis*, routinely cited for the South and Southeast of Brazil, was a misidentification of *G. isabellana* previously cited for Brazil as *G. lacinulata*. Of the analyzed samples two species remain unidentified. The use of molecular tools in the identification and separation of flattened Gracilariaceae proved to be very efficient and necessary, once morphological characters contribute little for the correct identification.

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THE ECTOCARPUS LIFE CYCLE IN THE FIELD SUPPORTS THE HYPOTHESIS OF

NICHE SEPARATION BETWEEN GAMETOPHYTES AND SPOROPHYTES

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The brown alga *Ectocarpus* has become a model for the genetic analysis of haploid diploid life cycles in the laboratory, but very little is known about the occurrence of different species and the succession of developmental stages in nature. We used a population genetics approach to determine which phases of the complex *Ectocarpus* life cycle are exploited under field conditions. We investigated the spatio-temporal distribution and ecological preferences of the species *E. crouaniorum* (Ecro) and *E. siliculosus* (Esil) in Roscoff (France). A total of 905 individuals were sampled along a shoreline gradient during three consecutive years. Isolates were cultured in the laboratory for subsequent genetic identification. Using molecular markers, sex markers and microsatellite loci we aimed to determine whether sporophytes and gametophytes are found at the same place and time or whether they occupy different ecological niches. Sporophytes of Ecro were found as microscopic, epilithic thalli year-round, while male and female gametophytes were only found as macroscopic thalli epiphytic on *Scytosiphon lomentaria* during spring. In contrast, all Esil isolates were identified as sporophytes, whether found as epilithic microscopic thalli during winter or as epiphytic macroscopic thalli on various macro-algal hosts from spring to summer. In order to test the generality of this pattern, we isolated 204 *Ectocarpus* strains from a distant region, Naples (Italy). The sampling was carried out in spring. Interestingly, Esil in Naples appears to occupy a similar ecological niche as Ecro at Roscoff; the Naples Esil macrothalli were mainly gametophytes epiphytic on *Scytosiphon*, while epilithic microthalli were mostly sporophytes. These data demonstrate that the niche preferences of gametophytes and sporophytes may not only vary between species but also between populations of the same species. Our findings support a model of niche separation for maintenance of the haplo-diploid life cycle.

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ALGAL TURF SCRUBBERS: PERIPHYTON PRODUCTION AND NUTRIENT RECOVERY ON A SOUTH FLORIDA CITRUS FARM

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There is a strong need to develop strategies that reduce nutrient loading to Florida's waters. The purpose of this study was to investigate the nutrient removing ability and growth rate of periphyton grown on an Algal Turf Scrubber (ATSTM) receiving a continuous flow (227 L min⁻¹) of water from an agricultural drainage canal. An experimental scale ATS was constructed (1.2 m wide x 234 m long) on a research farm operated by the USDA in Ft. Pierce, Florida. Over an 18-month period, PO₄-P, NO₃-N, NO₂-N and NH₄-N removal averaged 16%, 49%, 19% and 41%, respectively. On average, the entire flow-way yielded 5.5 g m⁻² day⁻¹ (range: 1-16 g m⁻² day⁻¹) of dry weight; however the upper 60 m yielded 11 g m⁻² day⁻¹ (range: 2-24 g m⁻² day⁻¹). Almost 50% of production occurred in upper 25% of the flow-way. Growth rate increased during the summer months. Harvested periphyton contained an average of 24.1% C, 3.8% N, 0.38% P, and 0.003% Si, but these percentages varied significantly. Bacillariophyceae dominated initially, but overall the ATS was dominated by filamentous Chlorophyta.

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THE GENUS *JAAGICHLORELLA* REISIGL (TREBOUXIOPHYCEAE, CHLOROPHYTA) AND ITS CLOSE RELATIVES: AN EVOLUTIONARY PUZZLE

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The genus *Chlorella* (in the traditional sense) is polyphyletic and belongs to at least twelve independent lineages of the Trebouxiophyceae and Chlorophyceae. Most of the aquatic species belong to

the genera *Chlorella* and *Parachlorella* (within the so-called *Chlorella*-lineage of the Trebouxiophyceae), or to *Scenedesmus* and *Mychonastes* (within the DO-group of the Chlorophyceae) according to phylogenetic analyses of the SSU and ITS rDNA sequences. In contrast to the aquatic species, the terrestrial strains investigated so far form a monophyletic lineage (*Watanabea*-clade) within the *Trebouxia*-lineage of the Trebouxiophyceae. To the *Watanabea*-clade belong several genera with *Chlorella*-like morphology (*Chloroidium*, *Heterochlorella*, *Watanabea*, *Kalinella*, and *Viridiella*). We studied 22 strains isolated from soil, bark, and artificial hard substrates, which have been traditionally identified as *Chlorella luteoviridis*. To clarify the taxonomical status and intrageneric diversity of this group, we used an integrated approach (molecular phylogeny of SSU and ITS rDNA sequences, secondary structures, DNA barcoding, morphology, and polyol production) including the ecological distribution and ecophysiological properties, which could provide survival strategies and successful development in extreme biotops. All strains investigated produce ribitol as osmolytic active substance, and showed a low phenotypic plasticity, but a surprisingly high genetic diversity, which could only be resolved in complex evolutionary models based on secondary structures. Based on these results, we re-established the genus *Jaagichlorella*, and proposed new species of *Jaagichlorella*, *Kalinella*, and *Watanabea*.

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A SURVEY OF AQUATIC ALGAL VIRUSES (PHYCODNAVIRIDAE) IN MT. PLEASANT MICHIGAN

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The field of aquatic algal viruses is a largely understudied area, especially for small freshwater ponds. This is problematic given their abundance and effects on aquatic ecosystem, especially on the microorganisms, both algae and bacteria, that make up approximately 95% of the biomass in the oceans and produce half of the accessible oxygen on Earth (Sandaa, 2008). It is estimated that viruses lyse approximately 50% of microorganisms daily, releasing

nutrients back into the environment (Sandaa, 2008). Along with adding nutrients back into the environment viruses affect the diversity and abundance of their host communities (Sandaa, 2008). Most algal viruses are double-stranded DNA viruses belonging to the family Phycodnaviridae, with genomes ranging from 100-560kb in length. The presents of Phycodnaviridae in 17 small freshwater ponds were examined using an established detection method of membrane filtration, acidification and PCR. The results showed that such viruses are common members of freshwater pond ecosystems.

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TRANSCRIPTOMIC ANALYSIS OF THE RED SEAWEED *LAURENCIA DENDROIDEA* (FLORIDEOPHYCEAE, RHODOPHYTA) AND ITS MICROBIOME

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The red seaweed *Laurencia dendroidea* is an important source of halogenated compounds exhibiting diverse potential pharmacological activities and relevant ecological roles. Despite the large amount of research describing the chemical composition of *Laurencia* species, the genetic knowledge regarding this genus is currently restricted to taxonomic markers. Host-microbe interaction is a driving force for co-evolution, but molecular studies of seaweed-associated microbial communities are still rare. We analyzed six transcriptomes from specimens of *L. dendroidea* sampled in three coastal locations of Rio de Janeiro, Brazil. In this work, we unveiled the genes involved on the biosynthesis of terpenes and explored the interactions between this host and its associated microbiome. Functional annotations revealed predominantly basic cellular metabolic pathways. Bacteria were the dominant active group in the microbiome of *L. dendroidea*, notably nitrogen fixing Cyanobacteria and aerobic heterotrophic Proteobacteria. Bacterial sequences were mainly related to glycolysis, lipid and polysaccharide breakdown, and also recognition of seaweed surface

and establishment of biofilm. Eukaryotic transcripts, on the other hand, were associated with photosynthesis, synthesis of carbohydrate reserves, and defense mechanisms. We provided the first transcriptomic profile of *L. dendroidea*, increasing the molecular knowledge from Florideophyceae. Our data suggest an important role for *L. dendroidea* in the primary production of the holobiont and the role of bacteria as consumers of organic matter and possibly also as nitrogen source. Furthermore, this seaweed expressed sequences related to terpene biosynthesis, including the complete mevalonate-independent pathway, which offers new possibilities for biotechnological applications using secondary metabolites from *L. dendroidea*.

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CYANOBACTERIA FROM TWO UNUSUAL HABITATS – HOT SPRINGS AND CAVES

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Cyanobacteria are the primary oxygen-evolving colonizer in any habitat of this Earth because of their high adaptability. Twenty-one species of cyanobacteria belonging to six heterocystous and three non-heterocystous genera were identified. Seven cyanobacterial strains belonging to four genera were isolated from three hot springs and two caves situated in four distantly placed states of India. pH, temperature and light were limiting factors of these two habitats. Studies on growth rates, biomass production and mode of nutrition were carried out using limiting factors like pH, temperature and light. Strains isolated from caves were slow growers. Heterotrophic and photoheterotrophic growth (light + DCMU) were also recorded in two hot spring strains and two cave strains. Cave strains showed better growth under photoheterotrophic conditions. Cyanobacterial responses to environmental changes such as temperature and light, focusing on the expression and function of molecular chaperones (HSPs) were also studied in order to better understand the mechanism of adaptation of these strains in such unusual habitats. Identification of these strains was validated using a polyphasic approach and the ‘Bacteriological Code’

involving morphology, ecology, life cycle and 16S rRNA phylogeny.

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PROBLEMS AND PROSPECTS IN FINDING THE DINOFLAGELLATE TREE OF LIFE

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Dinoflagellates are challenging organisms for phylogenetic study for several reasons. They are an ancient and monophyletic lineage that occurs in diverse habitats ranging from ephemeral puddles to freshwater lakes to estuaries to the open ocean, and have diverse trophic modes including parasitism, phagocytosis, myzocytosis, photosynthesis, or a mixture of these. Their organellar genomes are highly modified and lack genes useful for phylogenetic purposes, and their nuclear genomes are very large, with few, if any, single-copy genes. The ultimate goal is a phylogeny of dinoflagellates that reflected the full diversity of the lineage, and that incorporated multiple nuclear-encoded genes. We used a combination of morphological analysis, gene-targeted PCR, environmental sampling, and high-throughput sequencing (including of environmental samples). This work emphasized the great diversity of dinoflagellates, both photosynthetic and heterotrophic. High-throughput DNA sequencing methods (e.g., Illumina, 454) are highly promising for the study of both cultured and uncultured species, and flow cytometry can help with efficient analysis of natural communities. However, a robust and universal phylogeny of dinoflagellates remains a work in progress.

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THE ORGANELLAR GENOMES OF THE MESOPHILIC RED ALGA *GRATELOUPIA TAIWANENSIS* (HALYMENIACEAE, RHODOPHYTA)

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We have sequenced and annotated the plastid and mitochondrial genomes of the red seaweed *Grateloupia taiwanensis* S.-M. Lin & H.-Y. Liang (Halymeniaceae, Rhodophyta). The plastid genome comprises approximately 191 kbp and encodes 233 protein-coding genes, including 34 novel ORFs. After comparing this genome with other red algal chloroplast genomes currently available, we have determined a "core" set of approximately 140 genes, found in all red algae and thought to be essential for plastid function. The mitochondrial genome comprises approximately 29 kbp and encodes at least 21 protein-coding genes, all of known function. Additionally, we have found that the gene sequence coding for cytochrome c oxidase subunit I (*coxI*) includes a Group II intron.

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STEPS TOWARDS THE DEVELOPMENT OF A BENTIC DIATOM INDEX IN THE REEF ENVIRONMENT

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Using benthic diatoms (Bacillariophyceae) as a tool for the evaluation of coastal water quality deserves particular attention since those microalgae are successfully used in freshwater for their bioindication capacity. In marine environment, the main asset of benthic diatoms is to grow in all types of ecosystems from temperate to tropical areas. Steps toward a marine diatom index for a routine use in biomonitoring consist in: i) developing a standard sampling protocol; ii) defining species sensitivities; iii) choosing indicator species and metrics. In an eight weeks experiment in Martinique (FWI), we collected biofilm on three types of artificial substrates, placed vertically at three meters depth. Fives sampling sites were chosen to characterize a large range of water chemistry and morphological conditions. A mature community, represented by a high diatom density and diversity,

was reached after five weeks colonization; we observed no effect due to the nature of the substrate. Further work consist of the definition of species optimal growth conditions, with a non-obvious relation between species densities and nutrients concentrations due to their quick consumption by organisms, particularly in coral reef environments.

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EVIDENCES FOR THE EXISTENCE OF TWO CRYPTIC SPECIES IN THE PYLAIELLA LITTORALIS COMPLEX (ECTOCARPALES, PHAEOPHYCEAE)

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During the last century, many authors have described a multitude of species, varieties or forms in the genus of *Pylaiella* according to their morphology, their life history and/or their ecology; nevertheless no clear consensus has been reached. In the present study, we investigated the occurrence of cryptic species in *Pylaiella littoralis* complex along the Brittany coast using DNA barcoding, multilocus and population genetic analyses. Spatio-temporal sampling was conducted to detect genetic variations among populations as well as possible phenological difference between populations. We sampled 180 individuals from 8 sites located along the Brittany coast between Saint-Malo and Quiberon (450 km) from March to October. Two genetic entities were clearly distinguished based on mitochondrial sequences showing a minimum of 2.2% divergence between them. These two entities were also highly genetically differentiated based on microsatellites loci suggesting that they may correspond to putative cryptic species. In addition, a strong geographical clustering was observed among populations within each of these two taxa. Instead the analysis of chloroplast and nuclear sequences revealed a single group which may be explained by a recent differentiation of the two taxa. These two putative sibling species were found in the same environment; however their growths were shifted in time according to seasons. This phenological shift provides an ideal framework for exploring questions

concerning adaptive divergence as possible mechanisms of speciation.

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SEAWEED – CORAL INTERACTIONS IN A HIGH CO₂ WORLD: VARIABILITY IN OUTCOMES AND COMPETITIVE MECHANISMS

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Space competition between seaweeds and corals is an important ecological process structuring coral reef communities. It has been hypothesized that the competitive strength of seaweeds over corals will increase under projected human-induced ocean acidification scenarios, tipping the competitive balance in favour of the seaweeds. However, the variability of competitive outcomes, and the mechanisms by which seaweeds may outcompete corals under high CO₂ conditions are unknown. We conducted ocean acidification experiments in the Great Barrier Reef, Australia, and explored the roles of 1) seaweed allelopathy and 2) coral stress (caused by high CO₂) as possible mechanisms involved in the interactions. We found that competitive outcomes vary considerably depending on the species of seaweed involved in the interactions. The combination of allelopathy and elevated CO₂ conditions on the corals contributed to reduced coral photosynthetic efficiency and increased coral mortality. These results suggest that seaweeds will continue to overgrow and out compete reef corals under future ocean acidification projections.

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THE CRYPTIC DIVERSITY OF THE GENUS *ASPARAGOPSIS* (RHODOPHYTA, BONNEMAISONIALES)

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The genus Asparagopsis is known for being invasive in several regions of the world. Until now, two species have been formally described: *A. taxiformis*, originally described in the Red Sea and widely distributed across tropical and temperate zones, and *A. armata*, described from the western coast of Australia and displaying today a worldwide distribution, although preferentially in cold temperate regions. Previous molecular data reported 4 divergent mitochondrial lineages in *A. taxiformis* while no structure characterized *A. armata*. This study aimed at carrying out a detailed examination of the distribution of the previously described lineages over a global scale, with a special focus in New Caledonia. Sequencing results of mitochondrial, nuclear and chloroplastic markers revealed two major results: 1) A new lineage for *A. taxiformis* present only in New Caledonia, Kermadec Islands (North of New Zealand) and Australia (West) increasing again the list of cryptic taxa within this specific epithet. 2) Even more unexpected was the finding of a second lineage within the taxa *A. armata* in Australia (West) and Tasmania. This study exemplifies the importance of cryptic diversity in algal species.

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CYST FORMATION AS A VIRAL EVASION STRATEGY IN *HETEROSIGMA AKASHIWO* DURING INFECTION WITH HAV

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Heterosigma akashiwo belongs to a small group of harmful, bloom-forming algae (Raphidophyceae) that cause toxicity in sea urchins, oysters and finfish. Blooms are difficult to predict because the marine genera in this group have a two-phase life-history: an active vegetative phase and quiescent cyst phase. Unlike other raphidophytes, *Heterosigma* does not require a mandatory dormancy period before cyst germination, allowing it to quickly switch phases as nutrient, temperature and light conditions change. We observed cell death, cyst formation and a small number of surviving, free-swimming cells in *Heterosigma* cultures subsequent to viral infection with HaV. Surviving, vegetative cells were immune to future infections with HaV, suggesting HaV-resistance. Cyst formation was a significant outcome

of viral infection but these cells were still susceptible to the virus after germination, implying a temporary evasion mechanism. Cultures treated with a caspase inhibitor (z-VAD-FMK) prior to infection had fewer cysts and more cell death. Our evidence suggests that while a large majority of vegetative cells die during infection, many cells evade viral infection through encystment, a process possibly involving caspase-like enzymes.

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TEMPERATURE DEPENDENT MODIFICATIONS OF THE GALACTOLIPID PROFILES OF *HASLEA OSTREARIA* AND *PHAEODACTYLUM TRICORNUTUM*

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We have previously determined that the chloroplast membrane galactolipid profile of the “blue” diatom *Haslea ostrearia* contains primarily C18/C16 (sn-1/sn-2) and C18/C18 forms of mono- and digalactosyldiacylglycerol (MGDG and DGDG, respectively). We also examined the galactolipid profile of the pennate diatom *Phaeodactylum tricornutum*, which contained primarily C16/C16 and C20/C20 forms of MGDG and DGDG. In this experiment, two species of pennate diatoms were grown at 20° and 30°C to determine what modifications were made to fatty acids attached to these photosynthetically important lipids. Previous studies on effects of temperature on fatty acid length and saturation have been performed numerous times on different organisms; however, to our knowledge no such study has been conducted on diatoms. At 20°C, *H. ostrearia* and *P. tricornutum* are rich in eicosapentaenoic acid (C20:5) at the sn-1 position and C16 fatty acids at sn-2 position of MGDG and DGDG. *H. ostrearia* also contained a large number of MGDG and DGDG forms with C18 fatty acids at sn-1 whereas *P. tricornutum* contains primarily C16 fatty acids at sn-1 of its other MGDG and DGDG forms.

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TAXONOMY OF THE GENUS *AURANTIOCHYTRIUM* (LABYRINTHULOMYCETES)

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Labyrinthulomycetes, the ubiquitous marine heterotrophs, are the candidates for commercial production of polyunsaturated fatty acids such as DHA and squalene. Especially the members of genus *Aurantiochytrium* are promising in the high productivity. Presently this genus comprises two species, *A. limacinum* and *A. mangrovei*. However, it has not been investigated whether the taxonomy of the species corresponds to molecular phylogeny, because the ex-type strain of *A. mangrovei* had been lost. We isolated the strains from Goa, India, the type locality of *A. mangrovei*; the morphological features of strain SEK-414 corresponded to the diagnosis of this species. The molecular phylogeny of 18S rDNA clearly shows that the strains of *Aurantiochytrium* separate into three independent monophyletic groups. SEK-414 and IFO-32693 (ex-type of *A. limacinum*) are divided into two groups, whose strains show the characteristic features of *A. mangrovei* and *A. limacinum*, respectively. On the other hand, the strains of the third group have the distinguishable morphological features in zoospore formation and amoeboid cell motility from two described species. In conclusion, we propose a new species of *Aurantiochytrium* for this group.

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SPECIES DIVERSITY OF INTERTIDAL MACROALGAE IN NORTHWEST COAST OF YELLOW SEA: USING MORPHOLOGY COMBINED WITH DNA BARCODING

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Although macroalgae in the intertidal zone are important biologically, ecologically and sociologically, species diversity of intertidal macroalgae along the coast of Yellow Sea has been hardly investigated since the end of the 1980's. Using DNA barcoding to assist morphological identification, investigations were carried out in the northwest coast of Yellow Sea since April 2011. Related reference collections, including herbarium and slices of detail structure, were made at the same time. Until the end of 2012, a total of 808

samples were collected, covering 5 orders, 6 families, 9 genera and 18 species of green algae (Chlorophyta); 7 orders, 19 families, 47 genera and 59 species of red algae (Rhodophyta); and 9 orders, 10 families, 13 genera and 17 species of brown algae (Phaeophyta). DNA barcoding greatly helped species identification, especially with morphologically similar species, confirmed that some species are identical in molecular concept, and found some new phylogenetic relationships. However, due to drawbacks of each applied marker and the incomplete gene database in Genbank, at least two markers are recommended as mutual complement. Above all, traditional methods based on morphology are still indispensable. Generally, the diversity of intertidal macroalgae presented high seasonal variation, and evidently had decreased according to previous literature. However, cryptic species or invasive species which have never been recorded in this area also emerged during our study. The activity will contribute to baseline data on coastal macroalgae in northwest Yellow Sea, knowledge on habitat types and typically associated macroalgal flora, and the discovery of cryptic and invasive species.

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DRAFT GENOME SEQUENCES OF *SACCHARINA JAPONICA*

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Saccharina japonica has received increasing attention due to its primary productivity, edible human food, and eco-systematic contribution. Since the introduction into China in the late 1920s from Japan, this intensively selected kelp has gradually evolved into the maricultural backbone in China, and so far more than 10 cultivars have been applied in the production. However, its biological processes and genomic architecture are yet to be understood. Here we report the draft genome sequence of a wild *S. japonica* strain using next-generation sequencing platform. Two 180 bp and two 3K insert libraries were sequenced, which generated approximately 90-fold sequencing reads, with sequencing depth of 30X and 15X, respectively. The assembled genome is about 550 Mb,

approximately contains 280 Mb (50% of the assembly) of repetitive sequences and characterizes with a heterozygosity rate of 0.65%. Preliminary genes prediction showed that there are over 9600 genes in *S. japonica* genome. The draft genome sequence is expected to facilitate the understanding of the metabolic pathways, genetic adaptation and domestication through human selection, and improvements of good-quantitative varieties in kelp.

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TRADE-OFFS OF SEXUAL AND ASEQUAL LIFE CYCLES IN *MASTOCARPUS PAPILLATUS*: HOW COMPLEX LIFE CYCLES CONFER ECOLOGICAL ADVANTAGE IN CHANGING ENVIRONMENTS

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Species with complex life cycles serve as models to test hypotheses of conflict or cooperation between the stages of a life cycle (e.g., sexual versus asexual, gametophyte versus sporophyte). The conflict between sexual and asexual reproductive modes is hypothesized to be resolved through poor performance of asexuals caused by accumulated mutations and low genetic diversity relative to sexual counterparts, relegating them to peripheral, often marginal habitats. Yet, in *Mastocarpus papillatus*, asexual fronds are more abundant and distributed more widely than sexual fronds across tidal elevations in California in all habitats. Although the reproductive effort of asexuals is less than that of sexuals during peak reproduction, asexuals reproduce throughout the year and output is less sensitive to environmental variation. Surprisingly, genetic diversity in asexual lineages of *M. papillatus* is comparable to that in the sexually reproducing lineage at microsatellite loci tested thus far. These data suggest that the sexual and asexual life cycles of *M. papillatus* may not be in conflict, but represent an adaptive combination for ecological success.

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SENESCENCE IN *CHLAMYDOMONAS* AND THE DETERMINANTS OF LONGEVITY IN BATCH CULTURE

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In batch culture, microalgae such as *Chlamydomonas reinhardtii* undergo predictable changes in growth rate as nutrient levels decline over time. Previous work from my lab (Humby et al. 2013, *J. Phycol* 49, 389-400) examined the molecular changes that occur as *Chlamydomonas* cultures age in stationary phase and proposed the hypothesis that these biochemical changes are an attempt to prolong survival until conditions improve. Stationary phase is a complex interaction of nutrient limitation, cell density effects, and cell age where the conditions experienced affect long-term survivability of the culture. Limiting gas exchange is a key effector of longevity in stationary phase where the age-related accumulation of starch is inhibited. This lack of a carbon sink likely contributed to the observed negative correlation of light-intensity with longevity. In stationary phase there is a light intensity-dependent decline in longevity that is likely due to the production of reactive oxygen species that cause cell damage leading to death, in line with proposed age-related effects in animals. This work will discuss these effects and other determinants of longevity in microalgae and explore the strategies used to enhance culture survival.

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FEAMAINN: FROM FOOD TO FUEL – CURRENT PHYCOLOGICAL RESEARCH IN IRELAND

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In Ireland, seaweed (*‘Feamainn’* in Irish) has been used for many generations to improve poor soils and provide incomes for rural coastal families in areas with few other economic advantages. Phycological research has always gone hand in hand with the development of the Irish seaweed industry, contributing to the knowledge of sustainable harvesting practices and the

valuable polysaccharides (carrageenan, alginate, etc.) that became so important in the last 60 years. This relationship continues, with the Irish Seaweed Research Group (ISRG) still assisting industry with applied research, which now includes the large-scale cultivation of *Alaria esculenta* and *Saccharina latissima* for food and biofuel. Taxonomic studies are combined with molecular screening techniques to identify bioactive compounds from native seaweeds for the development of new high-value products. Other projects, such as salmon-seaweed fish feed trials and Integrated Multi-Tropic Aquaculture research ensure collaboration with the wider aquaculture industry. Education and outreach activities are also of great importance to the ISRG, aimed at the phycological community (Algaebase), the seaweed industry (*‘how-to’* cultivation manuals) and the general public (SeaSearch seaweed identification workshops, and harvesting fact-sheets).

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IDENTIFICATION OF NOVEL BIODIVERSITY FOR MONITORING, SURVEYING, AND CONTROLLING CYANOBACTERIAL HARMFUL ALGAL BLOOMS IN SOUTHERN FLORIDA

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Cyanobacteria of the genus *Lyngbya* seasonally form extensive blooms in Southern Florida. The prolific production of bioactive secondary metabolites of many of these bloom-forming *Lyngbya* can be hazardous for humans and the natural environment. In our efforts to provide taxonomic clarity, we show phylogenetically that several of the most prevalent *Lyngbya* specimens in Southern Florida, in fact, represent novel cyanobacterial genera. Here we characterize and compare the ecology, morphology, evolutionary history, and secondary metabolism of these new taxonomic groups. These taxonomic descriptions are important for monitoring, predicting, or possibly controlling potentially harmful cyanobacterial blooms in Southern Florida.

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ENVIRONMENTAL CONTROL OF *PSEUDO-NITZSCHIA* SPP. BLOOM DYNAMICS AND PARTICULATE DOMOIC

ACID CONCENTRATIONS IN THE BAY OF SEINE (ENGLISH CHANNEL, FRANCE)

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Pseudo-nitzschia spp. are globally distributed marine planktonic diatoms. Some species of this genus are domoic acid (DA) producers and are therefore responsible for Amnesic Shellfish Poisoning (ASP). In France, several ASP events occurred in the Bay of Seine (English Channel) since 2004. In order to identify environmental factors controlling these toxic blooms, the dynamics of *Pseudo-nitzschia* spp. natural populations and concentrations of particulate DA were followed during one year. *Pseudo-nitzschia* spp. blooms occurred in spring and autumn. Particulate DA concentrations were not related to total *Pseudo-nitzschia* spp. abundance. This result confirms the importance of a species specific approach in *Pseudo-nitzschia* studies. Individual *Pseudo-nitzschia* species were identified in natural populations using transmission electron microscopy. *Pseudo-nitzschia* natural populations were characterized by an important species diversity and species specific bloom dynamics. High particulate DA concentrations were related to the presence of *P. australis* and particulate DA concentrations appeared to be also controlled by environmental conditions, especially nutrient concentrations. This study gives an insight into the relationship between *Pseudo-nitzschia* bloom phases and DA production in natural populations.

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INHIBITION OF LIPID ACCUMULATION IN MICROALGAE

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Triacylglycerides, energy storage compounds in plants and microalgae, are a preferred substrate for the chemical conversion of plant and microalgae-derived oil to biodiesel. When subjected to nutrient limitation or stress conditions, some microalgae accumulate large amounts of triacylglycerides. Due to their rapid growth and fatty acid content, microalgae have potential as

sustainable biodiesel producers. However, an improvement in oil producing capabilities is required to make microalgae biodiesel production economically attractive. In this project the fatty acid synthase inhibitor cerulenin was used to select cerulenin resistant mutants in *Chlamydomonas reinhardtii*. These mutants were characterized by quantifying the amount of lipid produced and by determining the fatty acid composition. Experiments are being conducted using both *Chlamydomonas reinhardtii* and *Nannochloropsis oceanica* in order to study the metabolic response to stress. Cerulenin is used to inhibit fatty acid synthesis and ethyl-2-bromooctanoate is used to inhibit triacylglycerol synthesis. Initial results show inhibition of neutral lipid accumulation in *C. reinhardtii* when cells are subjected to cerulenin as visualized by fluorescence microscopy and measured by flow cytometry. In contrast, neutral lipids accumulate in *N. oceanica*.

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ENVIRONMENTAL CONTROLS ON THE COCCOLITHOPHORE *EMILIANA HUXLEYI*

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The coccolithophore *Emiliana huxleyi* isolated from the Pacific Chatham Rise (New Zealand) was subjected to a series of diagnostic fitness response experiments in the laboratory. Photosynthesis, calcification, and growth response curves were constructed relative to different levels of pCO₂, temperature, irradiance, phosphate and nitrate. The experimental results showed insignificant effects of phosphate on all response variables measured, indicating high phosphate affinity of the *E. huxleyi* strain tested. Nitrate and light limitations significantly decreased growth, photosynthetic and calcification rates. The calcification to photosynthesis ratio was significantly higher under limiting irradiance, but not affected by nitrate concentration. The relative importance of these environmental factors to the physiology of *E. huxleyi* was ranked using canonical correspondence analysis (CCA). Temperature and irradiance were the main environmental factors controlling growth and photosynthetic rates; and nitrate was the main factor controlling calcification

rate. Based on the above observations, different combinations of two- and three-factorial experiments are underway to determine the interactive effects of relevant environmental factors on this ecologically-important species.

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MARINE MACROALGAE FROM THE PACIFIC OF CENTRAL AMERICA: PAST, PRESENT AND FUTURE

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The history of algal exploration through the Central American Pacific can be divided into three stages: 1) regional exploration and documentation, 2) species checklists by country, and 3) sampling for DNA-based diversity assessments. By the end of the second stage, species records represent 70% of the known number of algal species, and form the basis of a taxonomic list which has not been updated for more than 50 years. In a first attempt to document and update the previous account of marine algal species in the region, a checklist of the marine macroalgae of the region was published. A total of 410 species were regarded as currently valid names. For decades, it has been proposed that the marine flora of this region is scarce and inconspicuous. However the results of a series of recent studies aimed at establishing the true algal diversity in the region, indicate a different scenario. The taxonomic evaluation of three common genera (*Caulerpa*, *Dictyota* and *Padina*) based on morphology and gene sequence data has led to 3 range extensions and 2 new species, suggesting a promising future for the elucidation biodiversity in the region.

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ULTRASTRUCTURE OF *THALASSIOSIRA BIOCULATA* VAR. *RARIPRORA* IN THE ARGENTINE SEA SLOPE, A FIRST RECORD FOR THE SOUTHERN HEMISPHERE

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The Southwestern Atlantic Ocean is recognized as one of the most productive areas in the world and supports a very rich fishery. In order to study the biodiversity and seasonal dynamics of phytoplankton in Argentinean continental shelf and slope waters, a cooperative research project has been established (GEF- Patagonia, Argentina). As part of this project, both qualitative and quantitative samples collected during spring 2005, were examined using light and scanning electron microscopy. An extensive bloom of a small *Thalassiosira bioculata* var. *rariprora* was observed along shelf waters from 39-48°S, reaching concentrations up to 4.5×10^6 cells per liter and representing more than 90% of total phytoplankton abundance. *Thalassiosira bioculata* var. *rariprora* is a taxon initially analyzed by Gaarder and Hasle, but never validly published (Hasle personal communication). In this work its ultrastructure is analyzed and described for the first time and this variety is recognized as valid for science. The observed specimens are small (8.5-12.0 μm), solitary, rectangular in girdle view, and with numerous discoid chloroplasts. The valve face shows a single strutted process slightly subcentral, with an additional ring of strutted processes located between the valve face and mantle (3-4 per valve). The strutted processes are externally surrounded at their base by areolae with robust walls, and internally have four satellite pores and long tubular extensions. One labiate process is situated adjacent to the subcentral strutted process. The areolae pattern shows a tendency toward fasciculation (20-23 in 10 μm), sometimes with radial ribs. The cingulum is composed of numerous copulae with vertical rows of pores and a valvocopula with a similar ornamentation. The morphological features of these specimens are compared with related taxa, including an evaluation of the main morphological characteristics useful to differentiate them.

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SILICEOUS PLANKTON AND THE BIOGEOCHEMISTRY OF SILICON

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The concentration and availability of silicon (Si) influences the morphology and physiology of siliceous plankton, phytoplankton community structure, and the biological carbon pump. It has been hypothesized that over geological time the evolution and radiation of siliceous plankton may have been influenced by changes in the availability of Si as well as influencing the marine silicon cycle. I will summarize what is known about the availability of Si in the sea over geological time and how changes in the marine silicon cycle are related to observed macroevolutionary changes in the size and morphology of siliceous plankton, with a focus on Cenozoic diatoms and silicoflagellates.

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MOLECULAR SUPPORT FOR TEMPORAL DYNAMICS OF INDUCED ANTI-HERBIVORY DEFENSES IN THE BROWN SEAWEED *FUCUS VESICULOSUS*

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Grazing by the isopod *Idotea baltica* induces chemical defenses in the brown seaweed *Fucus vesiculosus*. A combination of a 33 day induction experiment, feeding choice assays and functional genomic analyses was used to investigate temporal defense patterns and to correlate changes in palatability to changes in gene expression. Despite permanent grazing, seaweed palatability varied over time. Controls were significantly more consumed than grazed pieces only after 18 and 27 days of grazing. Relative to controls, 562/402 genes were up-/down-regulated in seaweed pieces that were grazed for 18 days, i.e. when defense induction was detected. Reprogramming of the regulative expression orchestra (translation,

transcription), up-regulation of genes involved in lipid and carbohydrate metabolism, intracellular trafficking, defense and stress response, as well as down-regulation of photosynthesis was found in grazed seaweed. These findings indicate short-term temporal variation in defenses and that modified gene expression patterns arise at the same time when grazed seaweed pieces show reduced palatability. Several genes with putative defensive functions and cellular processes potentially involved in defence, such as reallocation of resources from primary to secondary metabolism, were revealed.

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GENOME SEQUENCE OF BIOFUEL CANDIDATE ALGA PROVIDES INSIGHTS INTO STRESS ADAPTATION

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In the search for renewable and sustainable energy sources, marine microalgae show great promise because of their ability to thrive in brackish or seawater. This reduces the competition for fresh drinking water and provides some natural crop protection. We sequenced the 13.2 Mbp genome (7,367 genes) of a highly robust and halotolerant, biofuel candidate green alga, *Picochlorum*. When compared to *Ostreococcus tauri*, a non-halotolerant green alga of similar genome size, transporter analysis reveals an abundance of transporters in *Picochlorum*. Many of these transporters may play a role in halotolerance and are related to sodium transport, sugar transport, and drug efflux. Evidence of horizontal gene transfer in *Picochlorum* was found for 33 genes, most of which are related to DNA repair, lipid metabolism, and carbohydrate metabolism. We propose that horizontal gene transfer may play a key role in the adaptation of *Picochlorum* to high salinity and a variety of other environmental stresses.

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A REVISED PHYLOGENY OF THE RED ALGAL FAMILY PEYSSONNELIACEAE (PEYSSONNELIALES)

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The generic concepts of the crustose family Peyssonneliaceae will be critically assessed in a revised worldwide phylogeny of the family on the basis of phylogenetic analysis of chloroplast-encoded *rbcL* and *tufA*, chloroplast 23S UPA and mitochondrial *cox1* sequences derived from 336 samples worldwide representing 172 species and twelve genera. Type of diagnostic vegetative and reproductive morphological characters will be documented. The significant contribution of crustose Peyssonneliaceae in the formation of rhodolith beds in the offshore hard banks of the northwestern Gulf of Mexico at 45-85 m depth will be highlighted.

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INFLUENCE OF NITROGEN AND PHOSPHORUS DEFICIENCY ON AKINETE AND HETEROCYST PRODUCTION IN THE CYANOBACTERIUM ANABAENA SP.

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The genus *Anabaena* is one of the cyanobacterial taxa that dominate the majority of Brazilian eutrophic reservoirs. The dominance of *Anabaena* could be explained by the production of special cells such as akinetes and heterocysts. The aim of this study was to verify the capacity of *Anabaena sp.* to synthesize akinetes and heterocysts under nitrogen and phosphorous deficiency. This study was performed using *Anabaena* cultures under controlled conditions and AA culture medium (Allen and Arnon, 1955). The cultures were submitted to three different conditions: Medium AA complete (Control), Medium AA without nitrogen and Medium AA without phosphorous source. The comparison among the three growth curves showed a decrease in cells growth with nutrients deficiency. The largest heterocyst's synthesis was observed with treatment without nitrogen, and the phosphorous deficiency promoted the highest akinetes

production. Trichomes submitted to treatment without phosphorus and nitrogen appeared well fragmented and producing clumps compared to control. Our results showed that the nitrogen and phosphorous deficiency can promote the increase of akinete and heterocyst synthesis and could explain one of the strategies of *Anabaena* survival under deficiency of these nutrients.

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SOME ASSEMBLY REQUIRED: THE GREEN ALGAL TREE OF LIFE

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A handful of green algal lineages have been studied intensively for decades. Phylogenetically, however, these represent a small fraction of the group's diversity and relationships among major clades remain unresolved. Most prominently, seven deeply diverging lineages are lumped into the paraphyletic Prasinophyceae, but even well-studied, taxon-rich clades contain taxa of uncertain placement. Additionally, new lineages are discovered as unusual habitats are surveyed and modern techniques employed. Morphological traits are informative in some groups but utterly misleading or lacking in others. Therefore, systematic challenges are best approached from a molecular phylogenetic angle. The GrAToL covers the phylogenetic breadth of green algae and plants, including many previously ignored taxa by compiling and analyzing large amounts of targeted single-gene and next-generation DNA sequence data. As we work towards resolving the green tree of life, we find unexpected diversity among algae with simple morphologies, but also surprising relationships in algae with different and complex morphologies. High-throughput methods revealed lineage-specific patterns in the evolution of organellar genomes, including rampant mitochondrial and chloroplast rearrangements, and deep transcriptome sequencing yielded hundreds of putatively informative nuclear homologs.

MORPHOLOGICAL AND MOLECULAR EVIDENCES SUGGEST A NEW COMBINATION FOR *LAURENCIA COELENTERATA* (CERAMIALES, RHODOPHYTA) FROM THE TROPICAL ATLANTIC OCEAN

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As part of an international project in collaboration, morphological and molecular analyses were carried out on *Laurencia coelenterata*, a small sized species of red alga found in the seagrass bed at Verraco Beach, Santiago de Cuba. A detailed taxonomical investigation conducted on these specimens has revealed vegetative and reproductive characteristics belonging to *Osmundea*, such as two pericentral cells per each axial segment instead four, tetrasporangia cut off randomly from cortical cells, instead tetrasporangia originated from a particular fertile pericentral cells, and filament-type spermatangial branches developed in a cup-shaped spermatangial pit. These characteristics are in disagreement with the original circumscription of the species. The phylogenetic position of the present species was inferred by analysis of the chloroplast-encoded *rbcL* gene sequences complemented by plastid 23S rRNA gene (UPA). The range of genetic variation found in the analysis also supports the new taxonomical assessment for *L. coelenterata*.

ARE THERE WIDELY DISTRIBUTED NONGENICULATE CORALLINE SPECIES (CORALLINALES AND SPOROLITHALES, RHODOPHYTA)? INSIGHTS FROM SEQUENCING TOPOTYPES OR TYPE SPECIMENS

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Recent molecular studies of nongeniculate corallines have implied that some widely distributed species are really different species passing under one name. However, only by sequencing topotype specimens, or, much preferred, type specimens themselves, can names be assigned unequivocally. A *psbA* sequence obtained from a topotype specimen of *Heydrichia woelkerlingii* (Sporolithales) from South Africa differs by over 8.7% from a New Zealand specimen under that name in GenBank. An *rbcL* sequence from type material of *Spongites yendoi* (Corallinales) from Japan differs by over 15% from specimens given this name in South Africa. An *rbcL* sequence from type material of *Mesophyllum erubescens* (Corallinales) from Isla Fernando de Noronha off Brazil is an exact match to only one of four species passing under this name from Brazil; it differs by over 8% in *psbA* from New Zealand specimens under that name in GenBank. We question all reports of nongeniculate coralline species said to be widely distributed based on anatomical/morphological features. Our DNA sequencing results strongly imply that the number of extant species of both geniculate and nongeniculate coralline algae has been substantially underestimated.

EVIDENCE OF CELLULOSE IN EXOPOLYSACCHARIDES ISOLATED FROM ATACAMA DESERT CYANOBACTERIA

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Cellulose is a raw material for the production of biofuels since its degradation provides glucose that can be fermented to ethanol. Six species of five genera of cyanobacteria have been shown to contain cellulose. Also, cyanobacteria from desert environments synthesize high levels of exopolysaccharides (EPS) as an adaptive mechanism against desiccation. Our

results suggest the presence of cellulose in cyanobacteria from the Atacama Desert and our goal is to demonstrate that some of these Atacama cyanobacteria may be new unconventional biofuel sources. The Atacama cyanobacteria isolates studied showed EPS content in the range of 4-9 g per liter of culture. These EPS were substrates for commercial cellulase rendering 340-420 micromoles of glucose per min in a two-enzyme (cellulase plus cellobiase) reaction system, indicating the presence of a cellulose-like polymer in those cyanobacteria EPS. We were able to show the presence of the cellulose synthase gene, a key enzyme for cellulose biosynthesis, in the genome of only two Atacama isolates. Supported by CONICYT/FIC-R 4603; Universidad de Antofagasta CODEI-5394.

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EXOPOLYSACCHARIDE EFFECT ON THE ISOLATION AND SCYTONEMIN CONTENT OF CYANOBACTERIA INHABITING HALITES FROM THE ATACAMA DESERT

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Evaporitic sodium chloride rocks (halites) are one of the most extreme habitats in our planet where the type of inhabiting microorganisms and their survival strategies can be addressed. Halites from the Atacama Desert are colonized by a non-culturable cyanobacterium of the genus *Halotheca*. The cyanobacterium uses exopolysaccharides (EPS) to tolerate long desiccation periods and we took advantage of this property during our isolation strategy. Morphology, integrity and slow division were maintained for at least five months on agar plates supplemented with commercial alginate and/or EPS from heterotrophic and phototrophic microorganisms isolated from various habitats in the Atacama Desert, Chile. However, increase in growth rate was not obtained under these improved conditions. Scytonemin diminishes the deleterious effects of ultraviolet light. In our cyanobacterium, the scytonemin to chlorophyll ratio was 15-20 times higher in epilithic cells than endolithic cells. Similar results were obtained using

total protein concentration. Supported by IANAS Seed Grant Fund to AG; Universidad de Antofagasta CODEI 5394 to BG.

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PHOTOSYNTHETIC PERFORMANCE OF THE OBLIGATE EPIPHYTE, *VERTEBRATA LANOSA* (RHODOPHYTA) WHEN ATTACHED OR DETACHED FROM ITS HOST, *ASCOPHYLLUM NODOSUM* (PHAEOPHYCEAE)

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Vertebrata lanosa is an abundant and obligate epiphyte of *Ascophyllum nodosum* that forms part of a complex and highly integrated symbiotic system that includes the fungus *Mycophycias ascophylli*. As part of ongoing studies to resolve interactions among species in the symbiosis, we evaluated photosynthetic processes both in situ and in the laboratory by measuring effective quantum yield (Fv/Fm) and electron transport rate of photosystem II. The primary question we addressed was as follows: Is the ecological integration of these species reflected in a corresponding physiological integration involving photosynthetic process? Accordingly, in laboratory culture we measured changes in quantum yield over a seven-day period in thalli of both species when cultured in isolation, and when cultured together either attached or detached from each other. While quantum yield of *A. nodosum* showed no significant variation among conditions, *V. lanosa* showed increasing quantum yield in the following conditions: (1) *V. lanosa* alone, (2) *V. lanosa* in the same culture, but not attached to *A. nodosum*, and (3) *V. lanosa* attached to *A. nodosum*. These results are consistent with a separate experiment where electron transport rate was reduced in *V. lanosa* cultured alone vs. attached to *A. nodosum*. Values for quantum yield measured in situ in fully moistened thalli were similar to those measured in the laboratory; however, slightly desiccated thalli exposed to air for several hours had greatly reduced Fv/Fm. These results provide concrete evidence of a physiological association of the epiphyte and its host that reflects the known ecology.

LAURENCIA NATALENSIS KYLIN (CERAMIALES): A NEW RECORD FOR THE ATLANTIC OCEAN

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We report for the first time the species *Laurencia natalensis* Kylin (Ceramiales) for the Atlantic Ocean. Once thought to be restricted to the Indian Ocean, the presence of this species in the Atlantic is confirmed in this study by molecular analysis of the plastid-encoded large subunit ribulose-bisphosphate carboxylase/oxygenase gene (*rbcL*). Samples were collected in the Paraguana Peninsula (Falcon), Venezuela in the intertidal zone during a collection made on January 2012. Specimens were preserved in formaldehyde for anatomical studies and in silica-gel for molecular analysis. After DNA extraction, the *rbcL* sequence was obtained using different primer combinations. An alignment (1,210 bp.) was made with the Venezuelan sequence and 71 taxa (from the Tribe Laurenciae) available in GenBank. Molecular phylogenetic analyses were performed using Bayesian Inference and Maximum Likelihood. A well supported clade was recovered (100% posterior probability) unequivocally placing our specimen as conspecific of *L. natalensis* from Africa. Based on the results of the phylogenies, we discuss some insights about the molecular phylogeny of the *Laurencia* complex with emphasis on its generic circumscription. Further anatomical studies are required to contribute to a comprehensive description of *L. natalensis*.

MOLECULAR EVOLUTION OF NITROGEN ASSIMILATORY PATHWAYS IN SELECT MARINE PRASINOPHYTES

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Nitrogen assimilation is a highly regulated process requiring metabolic coordination of enzymes and pathways in the cytosol, chloroplast, and mitochondria. Previous studies of prasinophyte genomes revealed that nitrogen transporters have a

complex evolutionary history involving vertical and horizontal transmission. Here we examine the evolutionary history of well-conserved, nitrogen assimilating enzymes to determine if a similar complex history is observed. Phylogenetic analyses suggest that genes encoding nitrate reductase and glutamine synthetase (GS) III in the prasinophytes evolved by horizontal gene transfer from a member of the chromalveolates. In contrast to GSIII (which is predicted to be localized to the mitochondria or chloroplast), GSII was found only in *Micromonas* and appears to function in the cytosol. Phylogenetic analyses place the *Micromonas* GSII in a larger chlorophyte/vascular plant clade; a similar topology was observed for the ferredoxin-dependent nitrite reductases from both *Micromonas* and *Ostreococcus*. Thus genes encoding the nitrogen assimilating enzymes in *Micromonas* and *Ostreococcus* have been differentially lost and also recruited from several different evolutionary lineages, suggesting that the regulation of nitrogen assimilation in prasinophytes differs from other green algae.

SULFUR: AN UNDERESTIMATED FOCAL POINT IN ALGAL EVOLUTION AND ECOLOGY

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During the Mesozoic Era, phytoplankton with chloroplasts derived from secondary endosymbiosis with red algae (algae of the red lineage) became prominent primary producers in continental shelf waters, succeeding an earlier biota in which green algae and cyanobacteria were more abundant. This transition occurred concomitantly with an increase in the sulfate concentration of seawater. We hypothesized that the progressive increase of sulfate availability facilitated the evolutionary transition in marine phytoplankton. To test this hypothesis we cultured a number of algal species grown at different sulfate concentrations. Overall, the algae of the red lineage appeared to be more sensitive to low sulfate availability. Also the organic and elemental composition of cells was affected by the sulfate concentration used for growth, affecting the interaction of phytoplankton with their grazers. Collectively, our

results suggest that increase in seawater sulfate concentration through geologic history may have facilitated the evolutionary expansion of phytoplankton of the red lineage, but probably not to the exclusion of other biological and environmental factors. In order to assess what is the mechanism behind these differential responses of phytoplankton, we are now studying the sulfate assimilation pathway in a cyanobacterium, a green alga, a diatom and a dinoflagellate. Although some obvious differences emerge among these organisms, the results are not easily interpretable with respect to the evolutionary trajectories of phytoplankton. However, the data obtained reveal unexpected features of sulfate assimilation. The ensemble of our results will be discussed with reference to ecology and evolution.

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CHARACTERIZATION OF THE INTERACTION MACROALGAE – CORAL IN THE ALACRANES REEF NATIONAL PARK, SOUTHERN GULF OF MEXICO

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Macroalgae outcompete with corals for space, which is promoted by nutrient loading, and reduction of herbivores. Information on interactions between macroalgae and corals is practically unknown in study site. This work documents those interactions in the Alacranes Reef National Park (ARNP). Data were collected in seven sampling sites within 5 quadrants along two 25 m transects at three areas: northwest, center and southwest. Algal cover by functional form, total coral cover, type of interaction (macroalgae/cyanophyta/coral), and state of living/dead tissue were recorded. Seven algae functional forms and 16 stony coral species were recorded overall, with the following percentage cover: algae (36%), living hexacorals (27%), calcareous rock (29%), and other benthic organisms (8%). Concerning major algal groups and functional forms distribution and cover we found several differences: At the Northwest zone, *Lobophora* spp. and *Dictyota* spp. were dominant altogether with *Montastrea* spp. At the central area, *Halimeda* spp.

were dominant with *Diploria* spp. and *Porites* spp. while at the southwest filamentous algae and cyanophytes were dominant with *Montastrea faveolata*. In some sites we found evidences of a direct contact coral-algae and senescent mortality and in the central area of ARNP, coral living tissue was bleached.

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OPTIMIZING MICROALGAL GROWTH IN WASTEWATER EFFLUENTS

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Microalgae can potentially produce 30 times more liquid fuel oil per hectare than terrestrial plants without competing for agricultural land. If microalgal production is carried out in wastewater, algal biomass production could outperform terrestrial crops while offering the prospect of effluent bioremediation. Production and sale of algal feedstock for biofuels could offset increased costs of wastewater treatment associated with new N and P reduction mandates. Algal production in wastewaters, however, presents a number of challenges. To be economical for biofuel feedstock, microalgae must be grown to high density (> 1.5 g dry weight/liter) at a rapid rate. Resistant algal species may be isolated from effluents to overcome potential pathogens, competitors, and predators. Micronutrients such as magnesium and iron can become in such short supply that growth is severely reduced, even with abundant N and P. Furthermore, microalgae use a hundred atoms of carbon for each atom of phosphorus. Ambient carbon dioxide at about 400 ppm is not sufficient to achieve a maximum growth rate. Carbon dioxide must be provided in the range of 25,000 ppm or greater.

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AN OVERVIEW OF EUTROPHICATION – INDUCED HYPOXIA AND COASTAL DEAD ZONES

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Eutrophication-induced hypoxia (EIH), reduction of dissolved oxygen (DO) to levels incompatible with survival of coastal populations of fish and benthic fauna, is a consequence of increased phytoplankton production that is decayed by microbial respiration. EIH also fosters shifts toward microbial dominated ecosystems in which sulfate reduction generates the poisonous gas hydrogen sulfide. The occurrence of EIH is linked to global increases in the number and persistence of coastal dead zones having reduced fisheries production and consequently is of societal concern. The strength of the association between coastal dead zones and anthropogenic fertilization of nearshore waters is indicated by geographic occurrence at sites where watersheds deliver large quantities of nutrients into coastal oceans and near major population centers, and by cases of documented recovery when fertilization rates have decreased, by reducing sewage effluents, for example. Although multiple factors, including global climate change, foster the occurrence of EIH, the key to reducing dead zones is to prevent the movement of fertilizers into coastal oceans, thereby reducing the over-production of organic C whose decay consumes DO.

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TECHNOLOGICAL APPLICATIONS OF FRESHWATER GREEN ALGAL MICROBIOMES

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Algae are known to perform globally significant biogeochemical functions. Recent observations reveal that algae also provide extensive substratum rich in oxygen and organic carbon for diverse epibiotic microorganisms that aid host growth and extend algal ecological functions in surprising ways, including methane oxidation and nitrogen fixation. We compared microbiomes derived from Roche 454 shotgun sequencing and 16S amplicon analysis for green algal taxa having ecological and/or evolutionary significance: A) the widespread, nuisance bloom-forming, periphytic chlorophyte *Cladophora* – recently characterized as an ecological engineer; and B) several streptophyte algae, known to be closely related to the ancestry of land plants, therefore revealing ancient

plant attributes. Methanotrophs, nitrogen-fixers, nitrogenases of diverse types, and vitamin synthesis pathway genes were present. Algae-associated methanotrophs may prove useful in technologies for converting methane to liquid fuels; streptophyte nitrogen-fixers might aid efforts to improve the N₂-fixation capacity of agricultural plants, and microbiome information can facilitate technological use of algal systems to safely remove nutrients from wastewater effluents, thereby improving environmental quality and at the same time generating industrially useful products.

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TRENTEPOHLIALES (ULVOPHYCEAE, CHLOROPHYTA) FROM COASTAL SOUTH CAROLINA

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The Trentepohliales is an order of algae that occurs in subaerial habitats and sometimes in symbiosis with lichenized fungi. The trentepohlialean algae are most abundant in warm humid climates, however they can be found in cooler temperate zones. Most ecological research with this order has occurred outside the continental United States with emphasis on free-living subaerial epiphytes. This research project investigated the diversity and ecology of subaerial and lichenized Trentepohliales from selected coastal forests of South Carolina. Species identifications were based on microscopic observations, culture collections and molecular sequencing of the chloroplast-encoded large subunit of the ribulose-1,5-bisphosphate/oxygenase (rbcL) gene. Several different species of free-living *Trentepohlia*, *Printzina*, and *Cephaleuros* were documented. Phycobiont diversity was explored among four distinct fungal classes which represented seven different genera of lichen forming fungi: [Arthoniomycetes (*Herpothallon*)], [Dothideomycetes (*Astrothelium* and *Trypethelium*)], [Eurotiomycetes, (*Pyrenula* and *Strigula*)] and [Lecanoramycetes (*Graphis*, *Phaeographina* and *Phaeographis*)]. The use of rbcL primers to amplify genetic sequences of this plastid gene has continued to aid in the phylogenetic analysis of this algal group. Only

recently have researchers begun to move toward revealing the rich diversity of this elusive order. In agreement with previous research, the results of this study indicated that taxonomic schemes produced from morphological characters previously used for classification are not congruent with phylogenetic trees inferred from gene sequence information.

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VARIETY OF LIFESTYLES – LIKENESS OF TRAITS: AN INTEGRATIVE CASE STUDY ON THE GENUS *COCCOMYXA* (TREBOUXIOPHYCEAE, CHLOROPHYTA)

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The genus *Coccomyxa* belongs to a group of green algae (Trebouxiophyceae), which plays an important role in various terrestrial and aquatic ecosystems. They occur as photobionts of asco- and basidiolichens, as endosymbionts in certain ciliates or free-living in aerial biofilms and as phytoplankton in aquatic habitats. In the present study we investigated more than 40 *Coccomyxa* strains from different ecosystems and lifestyles. To discover functional biodiversity of this common small green alga we combined molecular with morphological and ecophysiological data in an integrative approach. We focus on the ability to accumulate ribitol as major stress metabolite in free-living species and as important energy source in symbiotic associations, respectively. Phylogenetic results based on SSU and ITS rDNA sequences showed that strains of *Coccomyxa* belong to at least three independent lineages within the *Elliptochloris*-clade of the Trebouxiophyceae. Surprisingly, two of these subclades contain strains of different habitats (free-living and photobionts of lichens) demonstrating the versatility of the investigated algae. To discriminate *Coccomyxa* at species level, we used the secondary structures of ITS-2 rDNA sequences as DNA barcode marker.

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THE FUTURE OF SEAWEED CULTIVATION

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Industrial-scale seaweed cultivation has been instrumental to the globalization of the seaweed industry since the 1950s. The domestication of seaweed cultivars in the 1940s ended the reliance on natural cycles of raw material availability for some species. Efforts to cultivate domesticated seaweeds were driven by market demands that far exceeded the available supplies. The success of seaweed cultivation is unrivaled in mariculture, with 94% of annual seaweed biomass utilized globally derived from cultivated sources. In the last decade, phycological research has confirmed seaweeds as rich sources of potentially valuable, health promoting, compounds; resulting in a partial shift away from the production of commodities (food and hydrocolloids) towards the production of value-added seaweed biomass. Consumer demand will ultimately drive the development of a new seaweed industry based on functionality and efficacy. Most existing seaweed cultivars and current cultivation techniques have been developed for the production of commoditized biomass, and may not be optimized for the production of bioactive compounds of high value. The future of the seaweed industry will include the development of high value markets for functional foods, nutraceuticals, and pharmaceuticals. Entry into these markets will require a level of standardization, efficacy, and traceability that has not been demanded of seaweeds in the past. Both internal concentrations and composition of bioactive compounds can fluctuate seasonally, geographically, bathymetrically, and genetically even within individual species. History has shown that successful expansion of seaweed products into new markets requires the development of domesticated seaweed cultivation, but future demands put on a new industry based upon efficacy and standardization will require the domestication of new species, further selection of improved cultivars, a refinement of

existing cultivation techniques and improved quality control.

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MOLECULAR BASIS FOR COMPATIBLE SOLUTE ACCUMULATION IN CYANOBACTERIA AND RED ALGAE

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Changing or high concentrations of inorganic salts (salt stress) represent one of the main environmental factors determining the occurrence of organisms in different habitats. Salt stress includes at least two primary stress components: the high amounts of inorganic ions exhibit direct toxic effects and the high osmotic potential in the surrounding soaks water out of the cell abolishing the capability of growth. Therefore, the cellular salt acclimation includes the active export of inorganic ions and the accumulation of non-charged, low molecular organic compounds (compatible solutes) keeping water inside the cell. These acclimation strategies were studied among cyanobacteria with laboratory strains of different salt tolerance levels defining genes and proteins involved in the acclimation to high external salinities. In my talk, I will summarize the current knowledge and illustrate how mining of cyanobacterial genomes revealed novel insights in salt acclimation strategies among marine cyanobacteria. Eukaryotic algae also accumulate different compatible solutes to thrive in waters of different salinities, but the molecular basis of their syntheses is less well known. Applying our strategy to study cyanobacterial compatible solute accumulation, we were able to identify the gene for isofloridoside synthesis in the unicellular red alga *Galdieria sulphuraria*.

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A SELF-CLEANING SEAWEED: CELL DIVISION WITHOUT MITOSIS IN ASCOPHYLLUM NODOSUM

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Fronds of the seaweed *Ascophyllum nodosum* remove colonizing epiphytes by intermittently shedding parts of the surface into the aqueous environment. This shedding involves epidermal cells dividing without mitosis to produce an outer layer of small, anucleate cells which then detach in localized patches along with the colonizing organisms. Fronds were collected weekly between June and August to monitor frond growth, shedding and to collect tissue for microscopy. Hand sections of living fronds were used to establish a method for reliably permeabilizing the epidermal cells with fluorescent dyes to stain nuclei and other cell components for light, fluorescence and confocal laser scanning microscopy. Previously fixed and resin-embedded tissue was sectioned to examine cell size, shape and patterns of cell division in the epidermis. Weekly sampling revealed that shedding occurred throughout the summer. No specific pattern in the distribution of shedding at the tips of the fronds was detected. Thick sections of fixed and embedded fronds revealed some remnants of the previously shed layer on the thick outer cell walls of the popcorn-shaped epidermal cells. The red fluorescent DNA-binding dye propidium iodide was selected to track the location of epidermal cell nuclei during the shedding process. Fluorescence microscopy and confocal laser scanning microscopy confirmed that the shed layers seldom contained nuclei. Moreover, re-examination of electron microscope images of shed layers confirmed that the cells are incomplete. A typical shed cell consists of an outer cell wall and the adjoining side walls, but it is wall-less on the side where it detached from the underlying the epidermal cell layer of the frond. Shedding occurs continuously during the summer and efficiently removes adhering microorganisms through a modified form of cytokinesis that results in a minimal loss of cell contents.

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PHYLOGENY AND SYSTEMATICS OF THE UNICELLULAR GREEN ALGA CYLINDROCYSTIS (ZYGNEMATOPHYCEAE, CHAROPHYTA)

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Taxonomy of the polyphyletic genus *Cylindrocystis* is complicated by a lack of structural characteristics in the vegetative cells. Formation and ornamentation of the zygospores are important for the identification of species. However, zygospores are rarely encountered in field material and the true distribution of most species is poorly understood. To better understand taxonomy in the genus, we investigated dozens of strains from public culture collections as well as strains isolated from North America. Mating experiments were performed to obtain mature spores for identification. In addition, two genes were sequenced from each strain and subjected to molecular phylogenetic analyses. Strains with vegetative morphology consistent with the widely reported species *Cylindrocystis brebissonii* were found in multiple lineages within the phylogeny. In addition, new lineages of *Cylindrocystis* were discovered. Based on phylogenetic placement and reproductive characteristics, two species new to science were discovered.

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CONTRASTING STRATEGIES OF PHOTOSYNTHETIC ENERGY UTILIZATION IN ECOLOGICALLY IMPORTANT PICOEUKARYOTES

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The efficiency with which absorbed light is converted to net growth is a key property for estimating global carbon production. We previously showed that, despite considerable evolutionary distance, *Dunaliella tertiolecta* (Chlorophyceae) and *Thalassiosira weissflogii* (Bacillariophyceae) share a common strategy of photosynthetic energy utilization and nearly identical light energy conversion efficiencies. These findings suggested that a single model might be appropriate for describing relationships between measures of phytoplankton production. This conclusion was further evaluated for *Ostreococcus tauri* and *Micromonas pusilla* (Chlorophyta, Prasinophyceae), two picoeukaryotes with contrasting geographic distributions and swimming abilities. Nutrient-dependent photosynthetic efficiencies in *O. tauri* were similar to the previously-studied larger algae. Specifically, absorption-normalized gross

oxygen and carbon production and net carbon production were independent of nutrient limited growth rate. In contrast, all measures of photosynthetic efficiency were strongly dependent on nutrient availability in *M. pusilla*. This marked difference was accompanied by a diminished relationship between Chl:C and growth rate. Our results suggest that energetic requirements for motility may cause this difference and that a different model describing photosynthetic energy utilization is needed for phytoplankton with strong energetic demands for motility.

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DIFFERENCE OF GENE EXPRESSION BETWEEN THE MALE AND FEMALE GAMETES IN A BROWN ALGA, *SCYTOSIPHON LOMENTARIA*

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The fusion of isogamous gametes of *Scytosiphon lomentaria* is the most primitive form of mating. This system could be used to analyze differences between the male and female sexes in algae. Proteomes of male and female gametes were compared to isolate sex related proteins. 2-DE analyses showed that most proteins (~700 protein spots in total) were expressed in both gametes. Only 12 proteins were expressed as sex-specific, which is <1% of the total number of detected protein spots. The most reliable proteins from each gel were selected and sequenced. Male-specific protein was identified as a GTP-binding protein, which is known to be involved in signal transduction. Female-specific protein was identified as succinate-semialdehyde dehydrogenase (SSADHD), which is involved in energy metabolism pathway in mitochondria. The expression levels of the genes before and after fertilization were analyzed using q-PCR method. High-expression of both genes was observed in each sample before fertilization, whereas during the development of zygotes it was reduced. Mitochondrial gene and signal transduction related genes were analyzed using 454-pyrosequencing method. The role of sex-specific genes is discussed in this study.

MACROALGAL COMPONENT OF A NOVEL MARINE RECIRCULATING LAND-BASED INTEGRATED MULTI-TROPHIC AQUACULTURE SYSTEM

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In integrated multi-trophic aquaculture (IMTA), macroalgae may serve as the assimilative component to utilize nutrients from the system's animal wastes. A prototype of a new concept in land-based marine closed-system IMTA has been developed that uses a centralized filtration system to deliver controlled volumes of selected pretreated waste streams to each system component and potentially resolves water quality and flow distribution issues related to traditional serial-flow closed-system IMTA designs. Production and nitrogen and phosphate removal were compared during the past year in side-by-side comparisons (three 11.1-m² tanks per species), for two species of macroalgae, *Gracilaria tikvahiae* and *Ulva lactuca*; both have been in outdoor cultivation for decades and are capable of sustained productivities (ca. 25 g dry weight m⁻² d⁻¹). Both species had high productivity and nutrient removal rates, but after several months *Gracilaria* became increasingly epiphytized due to continuous high enrichment. *Ulva* outperformed *Gracilaria* at the elevated pH (>9) in the system. The next step is to refine culture conditions in the prototype with the goal of developing *Ulva* as the primary macroalgal species in this system.

WHEN HUNGRY, *KARENIA BREVIS* GOES TOXIC

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Karenia brevis is the dominant toxic red tide species in the Gulf of Mexico. It produces potent neurotoxins (brevetoxins [PbTxS]), which negatively impact human and animal health, local economies, and ecosystem

function. Field measurements have shown that cellular brevetoxins vary from 1–68 pg/cell but the source of this variability is uncertain. The carbon:nutrient balance hypothesis, which was originally developed for terrestrial plants, predicts that nutrient-limited growth will be accompanied by a diversion of fixed carbon into increased levels of carbon-based defensive compounds or structures. Consistent with this hypothesis, data from various harmful algal bloom species have shown that nutrient limitation increases cellular toxin content. Historically, however, the effect of nutrients on *K. brevis* toxicity has been controversial. This study was undertaken to examine the effect of nitrogen (N) and phosphorus (P) limitation of growth rate on cellular brevetoxins in geographically diverse *K. brevis* strains. N- and P-limited cells had 2- to 3-fold higher PbTx per unit of cell volume or cell carbon. The percent of total cellular carbon associated with brevetoxins (%C-PbTx) was 1-4% in N-replete cultures and 5-9% in the N-limited cells. Similarly, %C-PbTx in P-replete isolates ranged from 0.7 to 2.1% in P-replete cells, but increased to 1.6–5% under P-limitation. Because PbTxS are potent anti-grazing compounds, this increased investment in PbTxS should enhance cellular survival during periods of nutrient-limited growth. The %C-PbTx for all isolates was also found to be inversely related to specific growth rate, which is consistent with an evolutionary tradeoff between carbon investment in PbTxS and other grazing defenses, and carbon investment in growth and reproduction. These results suggest that N- and P-limitation are important factors regulating cellular toxicity and contribute to the adverse economic and human health effects caused by *K. brevis* blooms.

SEASONAL AND SPATIAL TRENDS IN AN EPIPHYTIC MACROALGAL COMMUNITY IN A MANGROVE BASIN FOREST

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Mangals provide hard substrates that host many species of macroalgae. These communities contribute significant amounts of fixed carbon to these systems. Trends in primary production, biomass, and stress tolerance have received

limited attention despite the prevalence of this community type in mangal intertidal zones. This study examines the variation in ecophysiology and biomass of epiphytes in a basin forest in Tampa Bay, Florida, over seasonal as well as horizontal (m) and vertical (cm) spatial scales. With increased distance into basin forest, pneumatophore density and height increased providing a greater amount of hard substrate for the attachment and growth of epiphytes. Biomass per pneumatophore surface area was lowest in winter and fall, increased in the spring and was highest in summer. Distribution of the biomass was spatially complex with variations driven by vertical and horizontal trends that altered with season. Chlorophyll *a* concentrations varied by season with peaks both in the summer in conjunction with the highest algal biomass and in winter when biomass was low but the community composition appeared to have a greater abundance of chlorophyte algae.

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SARGASSUM MUTICUM ON THE ATLANTIC COAST OF MOROCCO: NEW RECORD AND RISKS OF ITS POTENTIAL EXPANSION ALONG AFRICAN SHORES AND NEARBY AREAS

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A new brown macroalga was discovered in the shoreline of Doukkala (Casablanca – El Jadida) in November 2011. Later surveys discovered new populations in tide pools and shallow subtidal areas along the Moroccan Atlantic coast. The species was identified as the highly invasive *Sargassum muticum*, which was introduced into Europe along both the Atlantic and Mediterranean coasts from Far East Asia in the mid-1970s, in association with transfers of oyster spats. Since then, *S. muticum* has expanded its distributional range in the European continent and has been reported along its Atlantic shores from Norway to Portugal. The vector of introduction in Morocco was identified again as the transfer of oyster spats coming

from French Atlantic sites. This highly invasive species is already established in the central Atlantic coast of Morocco and may spread towards nearby areas, affecting both the structure and composition of benthic populations. Phenological and ecological data of *S. muticum* in the Atlantic Morocco new sites are described. Guidelines for prevention of further expansion of this allochthonous species along the African shorelines and nearby oceanic archipelagos are discussed.

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GENETIC DIVERSITY IN THE BROWN ALGAL GENUS CLADOSTEPHUS (SPHACELARIALES)

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The macroalgal genus *Cladostephus* (Sphacelariales, Phaeophyceae) is found world-wide along coasts in temperate regions. Four of its valid species are considered endemic in South America, whilst specimens from the rest of the world are currently attributed to a single, cosmopolitan species, *C. spongiosus* (Hudson) C. Agardh. However, comparisons of molecular markers from samples within the geographic range of *C. spongiosus*, including Europe, North America and the Southwest Pacific, suggest that at least three genetic entities are treated under this name, requiring the reinstatement of *Cladostephus* species which have been previously synonymised with *C. spongiosus* based on morphology.

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TRANSCRIPTOMIC ACCLIMATION IN SACCHARINA LATISSIMA

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Kelps, brown algae of the order Laminariales, dominate rocky shores of cold-temperate regions. The distribution of kelps is constrained by abiotic factors like light including UV radiation, and temperature.

Future global environmental changes could therefore have a potentially significant impact on geographic distribution patterns, vertical zonation, and primary productivity of kelp. We established a cDNA library of *Saccharina latissima* consisting of 400,503 ESTs, which were assembled to 28,803 contigs. Sporophytes of *S. latissima* were exposed in multifactorial experiments to combinations of light intensities, UV radiation and temperatures. Subsequently microarray hybridizations were performed to determine changes in gene expression patterns. *S. latissima* responds to abiotic stress with a multitude of transcriptional changes. Expression profile changes in several metabolic pathways included, e.g., carbohydrate metabolism, lipid metabolism, and amino acid metabolism. Additionally several stress responses known from green plants like induction of ROS scavengers and induction of HSPs could be found. Another critical component of the molecular acclimation mechanisms to excessive light in *Saccharina latissima* is the induction of vitamin B6 biosynthesis.

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THE GENUS *LITHOPHYLLUM* (CORALLINALES, RHODOPHYTA) IN ATLANTIC EUROPE: MOLECULAR DIVERSITY, SPECIES DELINIATION AND TAXONOMY

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Lithophyllum is the most diverse genus in the Order Corallinales and family Corallinaceae with 110 morpho-species. We analyzed the molecular diversity of this genus in Atlantic Europe using *psbA* and *rbcL* sequences obtained from new collections and type specimens. The molecular data were also combined with anatomical, ecological and distributional data. Type specimens of *L. incrustans*, *L. dentatum* and *L. hibernicum* were successfully amplified, resulting in 0% matches with names traditionally used for those species. *Lithophyllum incrustans* is distributed from North Atlantic Europe to the Mediterranean,

represented as a rhodolith-forming species, entirely subtidal and only rarely observed as a crust. Crusts currently called *L. incrustans* matched the sequence of the type of *L. hibernicum*, historically known only as a rhodolith-forming species. Finally, *L. dentatum* appears to be restricted to the Mediterranean Sea. Our results indicate that the diversity of *Lithophyllum* in Atlantic Europe has been underestimated and additional type specimens of European species of *Lithophyllum* need to be sequenced before we can fully apply species names with confidence.

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CELLULAR ORIGIN OF TERPENES AND CORPS EN CERISE IN THE RED SEAWEED *LAURENCIA DENDROIDEA*

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In *Laurencia dendroidea*, terpenes are primarily stored in organelles called *corps en cerise* (CC). These terpenes can be synthesized by two pathways, the mevalonate (in cytosol, ER or Golgi derived vesicles), and the non-mevalonate (within chloroplasts). Thus, we aimed to identify the cellular origin of CC and the role of mevalonate pathway in terpene synthesis. A cytochemical assay was performed to localize active 3-hydroxy-3-methylglutaryl-CoA synthase, from mevalonate pathway. To determine the role of Golgi apparatus (GA) in traffic and storage of terpenes/precursors, algae spores were treated with Brefeldin A, a GA destabilizing drug. Then, spores were observed by transmission electron microscopy. Immunofluorescence and transcriptomic analysis were performed to localize and identify bromoperoxidases. As main results, it was revealed the occurrence of vesicles containing terpenes precursors (mevalonate pathway), depending on GA activity. Peroxidases were localized inside the CC (with halogenated elements), indicating the occurrence of bromoperoxidases within CC. Bromoperoxidases sequences (cDNA) were identified in *L. dendroidea*. Therefore, we suggest that vesicles containing terpenes (from mevalonate pathway) merge to form the CC, where bromoperoxidases act in the final step of terpene synthesis.

MOLECULAR ASSISTED ALPHA TAXONOMY REVEALS PSEUDO-CRYPTIC DIVERSITY AMONG SPECIES OF *BOSSIELLA* (FLORIDEOPHYCEAE) IN CANADA

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A floristic survey of the red algal genus *Bossiella* was conducted using molecular assisted alpha taxonomy (MAAT) and comparison to historical type specimens. The MAAT approach uses gene sequence data to assess species diversity followed by additional study including detailed morphological observations to delimit species. Currently, four species are recognized in the genus *Bossiella* in the northeastern Pacific. During a genetic screen using a DNA barcode marker, mitochondrial cytochrome c oxidase subunit 1 (COI-5P), we found evidence of 19 species groups, 17 of which have ranges that extend into Canada. Due to the large number of species requiring taxonomic assessment, we focused this study on those species with dichotomous branching (*B. californica* and *B. orbigniana*). We describe a new species (*Bossiella* sp. nov.), return the subspecies *Bossiella californica* subsp. *schmittii* to species status (*Bossiella schmittii* (Manza) H.W. Johansen), and resurrect the species *Bossiella dichotoma* (Manza) P.C. Silva. This is the first paper in a series that will attempt to elucidate species diversity for the coralline algae of the northeastern Pacific by combining historical type material sequence data and contemporary collections.

CONTEMPORARY AND HISTORICAL DNA SEQUENCES REVEAL EXTENSIVE CRYPTIC SPECIES DIVERSITY IN THE GENICULATE CORALLINE GENUS *BOSSIELLA*

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As oceans become more acidic, calcifying coralline algae are expected to decline. Recent molecular analyses have revealed our inability to differentiate species morphologically, to quantify extant diversity, and thus to detect shifts in species abundance in the future. Using morphological measurements, contemporary DNA sequences, and historical sequences from type specimens, we characterize the geniculate coralline alga *Bossiella plumosa*, the generitype of *Bossiella*, plus eight additional species that are often confused with *B. plumosa*. DNA extracted from the generitype of *Pachyarthron*, a genus traditionally confused with *Bossiella*, resolves in the genus *Corallina*. We recognize *B. frondifera*, a species previously placed in synonymy under *B. plumosa* but genetically distinct. We describe a *B. plumosa* species complex, consisting of four genetically distinct but morphologically indistinguishable entities. We transfer *Corallina frondescens* to *Bossiella* based on clear generic affinity. Finally, we describe three new *Bossiella* species that are likely lurking under the name *B. plumosa*. Our results highlight the fact that we cannot rely solely on morphology to differentiate coralline algal species and that we have underestimated the diversity of corallines worldwide.

PITFALLS OF THE GENERAL MIXED YULE-COALESCENT MODEL FOR AUTOMATIC SPECIES DELIMITATION IN PROTISTS

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The GMYC model has been proposed previously as a possibility for automatic species delimitation in metazoa. As an advantage over other methods it was emphasized that no prior knowledge about biogeographic distribution would be required. Studies have been published using GMYC in several animal groups, in macroalgae and even in bacteria. GMYC merges two models into one, the Yule process (speciation) and coalescence (intraspecific variation), to identify in a maximum likelihood approach a

threshold between the two processes and to set species limits. Since partial nuclear LSU rDNA has been proposed previously as a DNA barcode marker, a data set of sequences (approx. 900 nt) from morphologically and genetically characterized *Cryptomonas* species is used here to demonstrate problems inherent in this method, but also inherent in protists.

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NITROGEN BIOGEOCHEMISTRY AND DIAZOTROPHIC CYANOBACTERIA: LESSONS LEARNT IN AN AUSTRALIAN COASTAL LAGOON SYSTEM

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Fertilizer production and fossil fuel burning have greatly increased the amount of bioavailable nitrogen entering water bodies, leading to increased eutrophication, algal blooms and coastal water 'dead zones'. Nitrogen is a dynamic and transient element that has a major influence on phytoplankton growth and population dynamics, and, conversely, phytoplankton has a major influence on the nitrogen cycle. This influence is most dramatically illustrated by the nitrogen fixing capacity of diazotrophic cyanobacteria. A cyanobacterial bloom introduces vast amounts of nitrogen into the water, which is then cycled through the food web and eventually removed via sequestration or denitrification. The toxic N-fixing cyanobacterium *Nodularia spumigena* (Mertens) occurs in brackish water and blooms sporadically in the Gippsland Lakes in southeast Australia. These blooms are strongly influenced by catchment inflows in winter-spring and subsequent biogeochemical cycling of nitrogen and phosphorus through the lake sediment. During a bloom, newly fixed nitrogen moves through the food web not through direct consumption by zooplankton, but rather through consumption of bloom-associated bacteria. 200 years of European settlement have had a major impact on bloom occurrence in these lakes.

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ADAPTATION STRATEGIES OF TERRESTRIAL STREPTOPHYCEAN

GREEN ALGAE TO DESICCATION AND UV IN ARCTIC-ALPINE HABITATS

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Green algae are widely recognized as aquatic organisms, however, several members of the Klebsormidiophyceae and Zygnemophyceae (Streptophyta) occur under terrestrial conditions. They grow either on rocks, bark of trees, in soils as biological soil crusts or in habitats that fall dry occasionally. Living outside aquatic conditions these green algae are regularly exposed to high ultra violet (UV) radiation and desiccation in their high alpine or polar habitats. We investigated molecular-taxonomically characterized *Klebsormidium* sp., as well as *Zygnema* sp. and *Zygonium ericetorum*. Upon experimental desiccation photosynthetic performance decreased to a minimum, but after rehydration initial levels were fully or partly gained, depending on the strain. High osmotic values (low osmotic potential) were measured in *Klebsormidium* sp. contributing to a pronounced water holding capacity leading to desiccation tolerance. In *Zygnema* sp. osmotic values were lower. Organelle integrity of chloroplasts and the nucleus was maintained during desiccation as investigated by TEM. In *Klebsormidium* sp. cell walls were particularly flexible and followed the shrinkage process, which allows maintaining a turgor pressure even under water loss. Chemical and immunological assays of the cell wall of *Klebsormidium* indicated, that the cross walls particularly contain callose detected by aniline blue test and with an antibody to (1-3)- β -glucan. In addition, the hemicelluloses (1-3, 1-4)- β glucan, (1-4)- β -mannan and galacto-(1-4)- β -mannan were detected. In *Zygnema* sp. mostly pectin-like carbohydrates were localized. High solar irradiation might also be a problem for aeroterrestrial algae. Experimental exposure of *Zygnema* sp. and *Zygonium ericetorum* to enhanced UV and PAR radiation did not lead to drastic ultrastructural and physiological changes. Large amounts of phenolics and hydrolysable tannins were detected in sun-exposed field samples of both species, which likely have protective capacities.

DNA BARCODING OF THE ORDER BRYOPSIDALES (CHLOROPHYTA)

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DNA barcoding has proven to be a useful tool for resolving taxonomic problems on imprecise delimitation morphologically and reaching a new discovery in macroalgae. The order Bryopsidales can be difficult to identify due to its very simple structure and high morphological variation. In Korea, 5 genera with 46 species of Bryopsidales are reported, but the taxonomy of each taxon still remains unclear. To confirm species diversity of Bryopsidales, especially *Bryopsis*, *Caulerpa*, and *Codium*, we amplified and sequenced *rbcL* exon 1 and *tufA* region known as useful marker for Chlorophyta. Both *rbcL* exon 1 and *tufA* have sequenced with highly successful rates (more than 90 %) and provided good resolution for identifying Korean Bryopsidales. These markers helped to correct wrong identifications of *Bryopsis* and *Caulerpa* species and to discover some cryptic species. We confirmed 9 *Codium*, 3 *Bryopsis*, and one *Caulerpa* species. One cryptic species of *Codium*, having crustose and prostrate habit, is detected as forming a monophyletic clade with Japanese specimens. The specimens identified as *Bryopsis plumosa* in the field are grouped with an Argentine specimen, but separated with a French specimen from collecting near the type locality. It is necessary to accumulate DNA barcoding data, such as *rbcL* and *tufA* sequences, continuously in Bryopsidales to increase the accuracy of species delimitation.

DEEP (30 – 50 M) SAND-DWELLING MARINE DINOFLAGELLATES

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The seafloor, at a depth of 30-50 m, off the Satsunan Islands, in subtropical Japan, consists of coral reef sands. By culturing the sand samples from this area, a

diverse array of sand-dwelling dinoflagellates have been discovered. Here, we report on three new species from this particular habitat, one from each of the genera *Prorocentrum*, *Pyramidodinium* and *Testudodinium*. The *Prorocentrum* species is characterized by having an asymmetrical kidney-shaped cell, just like *P. panamense*. It is, however, distinguished from the latter by having a different type of surface ornamentation. *Pyramidodinium* sp. is similar to the type species, *P. atrofusum*, in having a pyramid-shaped non-motile form, but differs by possessing longer spines. *Testudodinium* sp. possesses a typical morphology for the genus, but it can be distinguished from other species by lack of dorsal ribs and by forming an unusual floating mass of non-motile cells. Molecular phylogenetic analyses based on SSU rDNA revealed that the assignment to genus of each species was correct. In addition, the distinction between them and their nearest relative by virtue of morphology was confirmed at the molecular level.

MACROMOLECULAR COMPOSITION VARIES SIGNIFICANTLY BETWEEN GENETIC CLADES OF SYMBIODINIUM MICROADRIATICUM

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Understanding the mechanisms involved in the process of coral bleaching, and predicting its effect on the coral reefs of the world, has long been the focus of a vast amount of research. However there is much more information available on the photosynthetic processes rather than its associated biochemistry. This could be due to methodology; analysis of the macromolecular content of environmental organisms relies largely on time consuming assays that sometimes underestimate the concentrations of proteins, carbohydrates and lipids. This area of research could benefit greatly from the development of a fast, accurate and comprehensive screening technique that focuses on the system as a whole. FTIR Spectroscopy is a method whereby a “biochemical snapshot” of a system can be obtained with very little sample preparation and in a very short time. Here we demonstrate its ability to distinguish between clades of cultured *Symbiodinium microadriaticum* grown under identical conditions,

based on their biochemical composition. The development of various statistical models based on FTIR spectra data could result in an effective screening method to assess coral health, and add to current knowledge of the mechanisms of coral bleaching.

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WIDE-RANGE INVASIVE SUCCESS DESPITE SEVERE POPULATION GENETIC BOTTLENECK: A STUDY CASE OF RED SEAWEED *GRACILARIA VERMICULOPHYLLA*

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Introduced seaweeds have caused detrimental impacts on marine environment and ecology. Based on extensive specimen collection (125 localities, 608 specimens), we compiled mtDNA Cox1 data of the invasive *Gracilaria vermiculophylla*, a red seaweed reported to invade North American and European coastlines from Asia-Pacific during the past few decades, aiming to understand the possible invasion pathways and population genetic divergences. Genetic analyses indicated two regions as the most probable sources for the global invasion, south coast of South Korea, and Japan. Biogeographic distributions of unique haplotypes reveal six introduction routes as follows: three primary routes from Japan to Northeast Pacific, West Atlantic, and East Atlantic, respectively; three additional routes representing separate introductions from the Far East, Russia to the Northeast Pacific, West Atlantic and East Atlantic. In addition, Virginia, USA, and Brittany, France, likely served as the invasive bridgehead and played a crucial role in shaping the range expansion via secondary introduction. We also illustrated that invasive populations of *G. vermiculophylla* underwent a severe population bottleneck effect, with extreme low genetic diversity in most of the secondary invaded areas.

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METABOLOME ANALYSIS OF THE GAMMA-GLUTAMYL-CYSTEINE SYNTHETASE OVER-EXPRESSOR

***CHLAMYDOMONAS REINHARDTII* UNDER HIGH- AND LOW-LIGHT CONDITIONS**

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Microalgal TAG is expected for a next-generation biofuel feedstock, and *C. reinhardtii* has become a model for understanding mechanisms of TAG-accumulation. Oil-rich green algae generally accumulate starch and TAG under nutrient deficient or other stress conditions, but they rapidly grow with minimal storage materials under normal conditions. However, the γ -glutamylcysteine synthetase over-expressor strain (*GSH1 ox*) of *C. reinhardtii* accumulates starch during growth phase under nitrogen sufficient conditions. In this study, *GSH1 ox* and its parent strain CC-503 were autotrophically grown in flat-flasks with aeration. Continuous illumination of high (180 $\mu\text{mol}/\text{m}^2/\text{s}$) – or low (30 $\mu\text{mol}/\text{m}^2/\text{s}$) – light was supplied for culture. Under both conditions, metabolome (mainly ionic metabolites and glycerolipids) analyses by using capillary electrophoresis- and liquid chromatography-mass spectrometry were performed for exponentially growing cells. There were significant differences between two strains in amounts of TAGs, glycolipids and pigments. The other metabolic profiles between two strains under high- or low-light conditions were also determined.

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FLAGELLAR APPARATUS OF *SCHIZOCHYTRIUM AGGREGATUM* (STRAMENOPILES, LABYRINTHULOMYCETES)

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The Labyrinthulomycetes are characterized by an ectoplasmic net system originating from a unique organelle, the bothrosome. The stramenopiles are a major eukaryotic group, which includes not only photosynthetic but also heterotrophic organisms such as phagotrophs, osmotrophs, and parasites. These

heterotrophs, including the Labyrinthulomycetes, are highly diverse, branching at an early stage. Therefore, molecular phylogenies have not provided enough information about the relationships among these groups. In this study, we investigated the ultrastructure of *Schizochytrium aggregatum*, with a special focus on a comparison of the flagellar apparatus of zoospores to reveal its morphological affinity with other stramenopile groups. *S. aggregatum* shared the following morphological features with the phagotrophic flagellates, placidideans and bicosoecids: a paranuclear body located between the posterior part of the nucleus and the mitochondrion; dense vesicles beneath the cell membrane; R2 extended posteriorly and forming a split between the microtubules; a mitochondrion associated with R2. It is suggested that the labyrinthulomycetes evolved from a phagotrophic ancestor, and gained the abilities of decomposer through the evolution of an ectoplasmic net system with a bothrosome.

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NUTRITIONAL VALUE OF *ULVA RIGIDA*, *GRACILARIA CORNEA*, *LAMINARIA DIGITATA* AND *PALMARIA PALMATA* FOR EUROPEAN ABALONE

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Different species of macroalgae form part of the natural food for abalone; some of them are used as feed for the commercial production of these and other invertebrates. Since these macroalgae frequently belong to different families and environments, their biochemical composition can markedly differ among them. Thus, the type of macroalgae fed to abalone can significantly affect their growth, covering different proportions of the nutritional requirements of this animal. In the present study, the nutritional quality of *Ulva rigida* and *Gracilaria cornea* was compared to that of *Laminaria digitata* and *Palmaria palmata*. Protein, lipid, minerals, amino acid and fatty acid composition were compared among the different algae. These species were fed to *Haliotis tuberculata* for 176 days. Survival rates were very high (93-100%), regardless the macroalgae fed. However, feeding *P. palmata* significantly ($P < 0.05$) improved abalone

growth as well as feed intake, whereas *L. digitata* markedly reduce it in comparison to *Ulva rigida* and *Gracilaria cornea*. The high nutritional value of *P. palmata* was mainly associated to its protein and carbohydrate composition.

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PHYLOGENY OF SOME MICROSCOPIC DELESSERIAACEAN SPECIES INCLUDING *ACROSORIUM* (DELESSERIACEAE, RHODOPHYTA)

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16 *Acrosorium* species are listed worldwide. Over 30 samples of *Acrosorium* were investigated based on morphological and molecular analyses. Three major clades are recognized in our phylogenetic tree using *rbcL* sequence data: *A. polyneurum* clade, *A. yendoii* clade, and *Acrosorium ciliolatum*. Although previously only *A. ciliolatum* has been recognized by the presence of hooked branches, we observed different morphologies of hooked branches in *A. polyneurum* and *A. yendoii*. We also collected several microscopic blade species of *Cryptopleura*, *Delesseria*, *Erythroglossum*, *Haraldiophyllum*, *Hypoglossum*, *Martensia*, and *Schizoseris*. A total of 43 taxa were sequenced for *rbcL* and their phylogenetic analyses were inferred from molecular data sets and morphological evidence.

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AN EXPLORATION OF *SPONGIOCHRYSIS* (CHLOROPHYTA) AND THE SUBAERIAL MICROALGAL COMMUNITIES OF COASTAL HAWAIIAN *CASUARINA* TREES

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Spongiochrysis hawaiiensis, a single-celled subaerial alga, was first described as a genus and species in 2006 from a sample collected from the ocean-side bark of coastal Hawaiian *Casuarina* trees. Based on 18S (nuclear) sequence data, this species was placed in the Cladophorales, representing the first subaerial species described within the order. This previous study used

material collected directly from the field to generate their sequence results, since unialgal cultures were not successful. However, subaerial communities are complex matrices that may contain many taxa, and definitively linking the morphology of *S. hawaiiensis* with the sequence results obtained through these methods is difficult. Additionally, the community structure of Hawaiian subaerial communities has not yet been fully investigated and the diversity of these communities is unknown. A two-pronged approach was taken as a step towards answering these questions: first, cells of toptype material were investigated morphologically using light microscopy and isolated for single-cell PCR of the 18S V4 region. Secondly, the V4 region was sequenced from environmental samples using Roche 454 technology.

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BIOASSESSMENT OF PETROLEUM PRODUCTS' MIXTURES USING MICROALGAE

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The effects of mixtures of water soluble fractions of refined petroleum products on the growth of three microalgae were assessed for the purpose of establishing the potential toxicity or stimulation of the fuel oils. The refined petroleum products used were petrol, diesel and kerosene, at various concentrations of 0%, 25%, 50%, 75% and 100% WSF and the test algae were *Chlorella vulgaris*, *Desmodesmus quadricauda* and *Ankistrodesmus falcatus*. The treatments investigated were singles, double mixtures and triple mixtures. Comparatively, the interactive and combined effects of triple mixtures had the highest inhibition or growth retardation on the test algae, followed by the double mixtures while the single (no combination) was least. Of the single mixtures, there was growth stimulation of the test algae at lower concentrations and growth inhibition at higher concentrations. In conclusion, *D. quadricauda* and *C. vulgaris* were stimulated in many cases by the hydrocarbons studied.

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TEMPERATURE AND SALINITY EFFECTS ON DIFFERENT LIFE-HISTORY STAGES

OF SARGASSUM HEMIPHYLLUM VAR. CHINENSE IN HONG KONG SAR, CHINA

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Physiological responses of the holdfast (HFS), slow growing (SGS) and rapid growing stages (RGS) of *Sargassum hemiphyllum* to different temperature and salinity stresses were examined. For temperature, all stages showed a higher growth rate at a temperature 4°C lower than the field ambient. Adverse growth was observed at warmer temperature ($\geq 4^\circ\text{C}$ above ambient temperature) and most individuals died at extremely high temperature ($\geq 8^\circ\text{C}$ above ambient temperature). A retarded growth was observed at lower temperature ($\leq 4^\circ\text{C}$ below ambient temperature) and extremely low ($\leq 8^\circ\text{C}$ below ambient) for SGS and RGS respectively. Moreover, SGS individuals exposed to 18-26°C developed reproductive structures one month earlier than the field populations. For salinity, the optimal growth and survivorship were observed at normal salinity (35‰) for all stages and a decreasing trend of growth was observed with a drop in salinities. This study revealed variations in physiological tolerance at different environmental stresses across algal life-history stages and Hong Kong appears not to be the optimal place for them to grow. This species may not be able to tolerate large temperature increase and may shift its range of distribution northward should the trend of global climate change continues.

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MOLECULAR PHYLOGENY AND TAXONOMY OF THE EUTREPTIALES, A GROUP OF PHOTOTROPHIC EUGLENIDS WITH ANCESTRAL TRAITS

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The Eutreptiales and the Euglenales are the two main clades of phototrophic euglenids. Members of the Eutreptiales have two flagella that emerge from the canal. The Eutreptiales currently includes two genera, *Eutreptia* and *Eutreptiella*, separated only by the

relative lengths of the two emergent flagella. An improved understanding of the phylogenetic relationships within the Eutreptiales is important for elucidating the earliest evolutionary stages of phototrophic euglenids. Therefore, we performed detailed molecular phylogenetic analyses of several new 18S and 28S rDNA sequences using the mixotrophic *Rapaza viridis* as an outgroup in order to demonstrate that (1) *Eutreptiella* is paraphyletic and (2) the Eutreptiales consists of four main subclades. From these data, we recommend that *Eutreptiella* should be divided into three genera that are consistent with distinctive traits associated with the chloroplasts. This research also showed that the condition of two emergent flagella with unequal lengths is an ancestral trait not only for the Eutreptiales but also for photosynthetic euglenids as a whole. By contrast, a single emergent flagellum and two emergent flagella with equal lengths are traits that evolved secondarily within phototrophic euglenoids.

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SORUS FORMATION ON THE BASAL SYSTEM IN *AUREOPHYCUS ALEUTICUS* (LAMINARIALES, PHAEOPHYCEAE), AND ITS TAXONOMIC REVISION AT FAMILIAL RANK

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A second locality of the recently discovered kelp species *Aureophycus aleuticus* is found in St. George Island, the Pribilof Islands, Bering Sea. New molecular phylogenetic analyses using 8 chloroplast and mitochondrial genes showed that *A. aleuticus* was most basal among the so-called derived Laminariales (i.e., Alariaceae/ Costariaceae/ Laminariaceae/ Lessoniaceae) and sister to Chordaceae. The species was newly shown to form sorus of unilocular zoidangia on the surface of the basal system (discoid holdfast), a unique feature in Laminariales. The developmental process and anatomy of the sorus was similar to those of other derived kelps. Considering that *Aureophycus* is annual, and unlike any other derived laminarialean species, lacking any additional

structures such as mucilage grand cell/mucilaginous duct, pneumatocyst, branching of stipe, etc., *Aureophycus* retains most primitive morphological features of the derived laminarialean taxa. Because of the unique morphological features of the sporophyte morphology and independent phylogenetic position in the order, we propose a new family to accommodate the monotypic species.

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PHYLOGEOGRAPHY OF *BATRACHOSPERMUM GELATINOSUM* (BATRACHOSPERMALES, RHODOPHYTA) IN EUROPE

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The freshwater red alga *Batrachospermum gelatinosum* (L.) DC. inhabits streams of Europe and North America having been collected frequently on both continents. A study of this species showed little genetic variation throughout its range in eastern North America. This study was initiated to investigate its phylogeography throughout Europe. Specimens were collected from Finland, Estonia, Lithuania, Latvia, Poland, Great Britain, France, and Spain. Of the 37 specimens analyzed, there were 8 COI haplotypes that differed by 1-8 bp. In addition, ITS variation was surveyed and showed twice as many haplotypes and more base pair variation. A haplotype network of COI data showed most haplotypes were closely related but those from Spain were more distant. For the ITS data, the network had a star appearance and the specimen from Spain differ little from the others, but two haplotypes from Finland and Latvia were quite different. Compared to North America, there are more haplotypes present in Europe and the relationship among haplotypes is more complex. The geographic distribution of haplotypes in relation to glaciation events will be explored.

COMPARATIVE STUDY OF GALACTOLIPID COMPOSITION AND THE BIOSYNTHETIC GENE FOR DIGALACTOSYLDIACYLGLYCEROL SYNTHASE IN *VITRELLA BRASSICAFORMIS* AND *CHROMERA VELIA*

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Vitrella brassicaformis belongs to the recently erected phylum Chromerida, and is another close photosynthetic relative of non-photosynthetic apicomplexans described after *Chromera velia*. To date, no studies have been published regarding the biochemical study of its plastid lipids that could be useful for making a chemotaxonomic inference between *V. brassicaformis* and *C. velia*, and to the presumed plastid ancestor of chromerids in general. In this study, we used a positive-ion electrospray ionization/mass spectrometry (ESI/MS) and ESI/MS/MS approach to elucidate the fatty acid regiochemical composition of two major plastid lipids, monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG), in *V. brassicaformis* in order to provide a comparison with the forms of MGDG and DGDG as found in *C. velia*. Additionally, a genomic approach using RNA-seq data was used to identify the gene for digalactosyldiacylglycerol synthase, which encodes the enzyme responsible for converting MGDG to DGDG. *V. brassicaformis* primarily contained C20/C14, C20/C16 and C20/C18 fatty acid components attached to the glycerol moiety of MGDG and DGDG; these forms of MGDG and DGDG synthesized by *V. brassicaformis* are different and structurally more diverse than the previously identified C20/C20 forms that compose nearly the entirety of *C. velia*'s MGDG and DGDG. This biochemical difference is consistent with previously observed ultrastructural and pigmentation differences between *V. brassicaformis* and *C. velia*, and agrees with their classification under different orders, Vitrellaceae and Chromeraceae, respectively. However, phylogenetic

analysis of the DGDG synthase gene was consistent with a red algal origin.

A PUTATIVE NEW SPECIES IN THE FRESHWATER DIATOM GENUS *DIDYMOSPHENIA* (BACILLARIOPHYTA) IN A RIVER IN CONNECTICUT, U.S.A

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In 2011, a stalk-forming diatom closely resembling *Didymosphenia geminata* was observed blooming in the Farmington River, Connecticut. The mats formed by the bloom were washed away during two significant late summer storms, eliminating the algal blooms for 2011. Monitoring continued throughout 2012 with no observations of *Didymosphenia geminata* until late November when a bloom occurred. The cells were viewed with light microscopy and further examinations performed because of the unusual compressed morphology of the diatom. Partial sequences of the 18S rRNA gene were obtained using direct PCR of single cells isolated from an environmental sample. The cells walls used in the PCR reaction were then recovered and viewed using SEM for verification. This observed diatom produces copious mucilaginous stalks, forming mats that cover the substrate and has the triundulate frustule morphology similar to that of *D. geminata* although considerably smaller at a consistent range of 50- 55 µm. Morphological and DNA analyses support that this is a new diatom species, which will be described elsewhere.

DIETARY EFFECT OF ALGAL FEED ON THE GROWTH, CARCASS COMPOSITION AND PIGMENTATION OF *LABEO ROHITA* AND *CARASSIUS AURATUS*

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Feed has become the most important component of the culture system from the viewpoint of both fish production and cost. Due to the scarcity and escalating cost of fish meal algae are now considered as efficient feed ingredient due to its high nutritional values. A 12 week feeding trial was conducted to evaluate the suitability of four unconventional algal genera, viz., *Phormidium tenue*, *Nostoc ellipsosporum*, *Synechocystis pevalekii* and *Rhizoclonium fontinale* as fish feed supplement for *Labeo rohita* and *Carassius auratus*. Market available fish meal was considered as control feed. Algal biomass was mixed (40% for rohu and 70% for goldfish) with the control feed to formulate algae based value added feed. The proximate composition of feeds and experimental fishes were analyzed. The specific growth rate, feed intake rate and protein efficiency ratio recorded in both the experimental fish improved significantly when fed with algal meal. Carcass protein, lipid and glycogen content increased for the algal diet. Colorimetric and HPLC data showed increased amount of β -carotene and astaxanthin in goldfish. The new composite algal meal can serve as an alternate replacement to the costly conventional fish feed.

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CLONING *SPIROGYRA VARIANS* THIOREDOXIN H INDUCED BY GAMMA IRRADIATION AND ENZYMATIC CHARACTERIZATION

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Thioredoxins (Trxs) are small ubiquitous proteins containing a conserved active site, WCGPC, which reduces disulfide bridges in target proteins. Trx plays a role in a wide variety of physiological process by acting as a general disulfide reductase. Initially, Trx and the specific NADPH-dependent enzyme thioredoxin reductase (NTR) were purified from *Escherichia coli* as a hydrogen donor system for ribonucleotide reductase. However, Trx has not been isolated from other algal species, and is not as characterized as in bacteria and higher plants. Trxh is involved in multiple processes, with the best-documented function of Trxh being its role in reserve breakdown that sustains early seedling growth in

cereals. Furthermore, Trxh may be involved in cellular protection against oxidative stress, modulation of redox-dependent signaling cascades, and carbon and nitrogen metabolism. Thus, this protein is likely to be involved in antioxidant systems, signaling pathways, as well as developmental processes. Limited information is available on the expression and specific functions of algal Trx during oxidative stress caused by gamma irradiation. Radiation acts mainly through radiolysis of water molecules and induces the production of reactive oxygen species (ROS), which cause extreme oxidative stress in cells. In the present study, proteomic changes were examined in *Spirogyra varians* following gamma irradiation. The *S. varians* Trxh (SvTrxh) gene was isolated using internal primers based on the partial amino acid sequence from matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS). Characterization of SvTrxh revealed disulfide reduction activity and differential expression patterns in response to gamma irradiation. Moreover, the results from this study suggest a potential function of SvTrxh in protection of biomolecules from oxidative stress of gamma irradiation.

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COMPARATIVE TRANSCRIPTOME ANALYSIS OF THE REGENERATING PROTOPLASTS OF *BRYOPSIS PLUMOSA*

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The protoplasm extruded from the injured cells of the green alga *Bryopsis plumosa* can generate numerous new cells in seawater. The cell organelles aggregate rapidly in seawater and become covered with a gelatinous envelope within 15 min. A lipid-based cell membrane developed inside the gelatinous envelope about 12 h after wounding. The gene expression during the regeneration of cell membrane was studied using RNAseq and Microarray analysis. Three large EST databases were constructed using pyrosequencing. The profile of transcriptome dramatically changed over the time course of protoplast regeneration. GO analysis showed numerous stage-specific gene groups which are involved in various cellular processes including antioxidative activity, signaling process and

photosynthesis. Comparative analysis of ESTs databases using PESTAS software revealed groups of genes which were newly expressed during the regeneration of cell membrane. The genes involved in cellular lipid and polysaccharide catabolic process and glycerolipid biosynthetic process were expressed at 6h after protoplast regeneration. At 12h after protoplast regeneration, the genes involved in glycerolipid metabolic process and signaling pathways were specifically expressed. Microarray analysis showed the transcriptome profiles shifted from one stage to another. Bioinformatic methods used for adjusting consistency between RNAseq and microarray datasets were discussed. Results indicated that there are several specific signal pathways turn on and off at each stage of protoplast regeneration in *Bryopsis plumosa*.

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**MULTI-CROPPING SEAWEED
GRACILARIA TIKVAHIAE WITH OYSTERS
FOR NUTRIENT BIOEXTRACTION AND
SEA VEGETABLES IN WAQUOIT BAY,
MASSACHUSETTS**

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A native red seaweed, *Gracilaria tikvahiae*, was cultivated on an oyster farm to investigate the dual purposes of nutrient bioextraction and production of edible sea vegetables. *Gracilaria* was grown in the spring of 2012 to facilitate a first planting in June 2012. *Gracilaria* was planted at two sites (with and without oyster cages), at two depths (0.2 m and 0.6 m), at two stocking densities (20 g bundles planted every 10 cm and 20 cm), and at two harvest intervals (every 2 weeks and every 4 weeks). *Gracilaria* grew very well at both locations. Best yields per unit area have been found at the shallower depth (0.2 m), at high stocking density (10 cm) and at two-week harvest intervals. We estimated approximately 50 kg of N per growing season (June to October) can be removed by *Gracilaria* in a hypothetical one hectare farm with 3 m spacing between longlines. However, fouling (e.g., primarily tunicates) were a problem, especially when *Gracilaria* was cultivated longer than weeks.

Freshwater treatment helped to overcome the fouling problem.

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**COMPLETE MITOCHONDRIAL
GENOMES OF TWO GELIDIALEAN
SPECIES, *GELIDIUM ELEGANS* AND
GELIDIUM VAGUM (RHODOPHYTA)**

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Gelidium J.V. Lamouroux, the largest genus of the order Gelidiales, is distributed globally. The diverse uses of this genus as food, agar, biofuel and paper pulp have increased interests in its member species. Two agar-producing species, *Gelidium elegans* and *G. vagum*, are commonly found in Korea, China, and Japan. We sequenced and characterized the complete mitochondrial genomes of two *Gelidium* species using next-generation genome sequencing methods. Genome size and GC content of *G. elegans* and *G. vagum* were slightly different as 24,922 nt vs. 24,901 nt, and 29.5% vs. 30.4%, respectively. Forty-four genes of *G. elegans* were identified including 23 protein-coding genes, 2 rRNA genes, and 19 tRNA genes, while 43 genes including 23 protein-coding, 2 rRNA, and 18 tRNA genes were encoded in the mitochondrial genome of *G. vagum*. The gene synteny was very similar between *G. elegans* and *G. vagum*, but the trnH gene was absent in *G. vagum*. There were gene length differences in rrl, rps3, trnL, sdh2, sdh3, trnS, trnC, nad5, trnA, trnK, secY, rps12, and rrs, which were made genome variations between two species within the genus *Gelidium*. It will contribute to further study about red algal mitochondria genome diversity and evolution.

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**DISCOVERY OF CRYPTIC SPECIES IN
NEOSIPHONIA (CERAMIALES,
RHODOPHYTA) FROM KOREA BASED ON
MOLECULAR EVIDENCE**

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The genus *Neosiphonia* was established by Kim and Lee in 1999 and reported 30 species from the tropical to cold temperate regions. Species boundaries, based on morphological discontinuities and the typological species concept, have been considered problematic because of substantial morphological variation. In the past decade, molecular analyses have been applied to clarify the taxonomic status of red algal species. In Korea, approximately 12 species have been described based on morphological characteristics only. To confirm species diversity of the genus *Neosiphonia* from Korea, we sequenced the plastid protein-coding *rbcL* gene for species-level phylogenetic questions and mitochondrial COI gene for DNA barcoding. A total of eight *Neosiphonia* species, including three cryptic species recognized by molecular evidence: *N. japonica*, *N. harlandii*, *N. yendoii*, *N. sphaerocarpa*, *N. teradomariensis*, *Neosiphonia* sp1, *Neosiphonia* sp4, and *Neosiphonia* sp5. The three unidentified species are characterized by having multi-pericentral cells (over 4) and living in the subtidal zone. Molecular sequence analyses strongly supported that the genus *Neosiphonia* has cryptic species diversity from Korea.

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ULTRASTRUCTURAL STUDY OF FLAGELLAR APPARATUS AND ANALYSIS OF FLAGELLAR MOTION IN BROWN ALGAE

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Swarmers of heterokont algae have two morphologically and functionally heterogeneous flagella. In addition, the flagellar apparatus is complex, consisting of microtubular flagellar rootlets and several bands. These features are well conserved in heterokont algae and closely related to cytoskeletal organization and flagellar motility. In this study, we showed (1) ultrastructure of the flagellar apparatus and (2) the flagellar beat pattern of male gametes of brown algae. Ultrastructure of flagellar apparatus has been reported, but these data were from conventional TEM

observations. A detailed high-resolution map integrating all structural components of the brown algal basal apparatus was needed, and we decided to reinvestigate it using electron tomography. This technique allowed us to define connections and positional relationships between the structural components. Additionally, several unknown bands were found. Fertilization in some brown algae takes place by fusion of isomorphic gametes. Female gametes settled down on surface and release pheromone. Afterwards, male gametes are attracted to the female. Using high-speed video microscopy, we observed the flagellar motion of male gametes during fertilization.

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PHYSIOLOGICAL MECHANISMS IN PHOTOACCLIMATION OF THE GIANT KELP *MACROCYSTIS PYRIFERA* (LAMINARIALES, PHAEOPHYCEAE) ALONG A DEPTH GRADIENT

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The giant kelp *Macrocystis pyrifera* can grow up to 60 m long and may span the entire water column. Hence, the single sporophyte is exposed to a large vertical gradient of radiation quality and quantity. To cope with these differences in light conditions, *M. pyrifera* exhibits a high capacity and plasticity for photoacclimation. While the importance of photosynthetic pigments in photoacclimation is already known, the contribution of lipids (especially fatty acid composition) as well as phlorotannin and antioxidant concentrations is largely overlooked. To close this gap, we conducted a 14-day field study in the Bay of Coquimbo, Chile. During summer 2011/12 and winter 2012, apical tips of *M. pyrifera* sporophytes were tethered at 3 different water depths (0.2, 2 and 4 m). As expected, apical tips tethered near the surface represented the largest xanthophyll cycle pigment pool and de-epoxidation state (DPS), displaying acclimation to high-irradiance conditions. This photoprotection response was particularly pronounced in summer. The

different light climates in the 3 water depths resulted in slightly higher total lipid contents in summer than in winter.

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TAXONOMY OF THE GENUS *HERPOSIPHONIA* (CERAMIALES, RHODOPHYTA) FROM KOREA

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The red algal genus *Herposiphonia* is composed of about 50 species and is widely distributed throughout tropical and warm-temperate regions of the world. *Herposiphonia* is distinguished by morphological characteristics, such as the branching pattern, shape of determinate branches, number of pericentral cell, position of the cystocarp, and frequency and arrangement of the vegetative trichoblast. We confirmed species diversity of *Herposiphonia* on the basis of morphology and molecular analyses using plastid *rbcL* and mitochondrial COI genes. In the result of molecular analyses, *Herposiphonia* is distinctly separated by five species; *H. fissidentoides*, *H. insidiosa*, *H. parca*, *H. secunda*, and *H. subdisticha*. In the morphological study, the pattern of determinate (d) and indeterminate (i) branches is d/d/d/i in most species, except *H. secunda* having d/i. Shape of determinate branches is usually terete, but *H. fissidentoides* is ligulate. The number of pericentral cell is 6 to 8 in *H. insidiosa*, 10 to 12 in *H. subdisticha*, 14 to 16 in *H. fissidentoides*, and 8 to 10 in others. Vegetative trichoblasts are lack in most species, but *H. parca* and *H. secunda* have short ones at the terminal of determinate branches. We are conducting the research on taxonomy of *Herposiphonia* to confirm species diversity and phylogenetic relationships.

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OSMOREGULATION IN *CHLAMYDOMONAS REINHARDTII*

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The maintenance of water homeostasis (osmoregulation) is vital for all life. In many

freshwater microalgal contractile vacuoles (CVs) constantly expel excess water taken up due to the hypotonic environment. We have investigated the physiology and molecular cellular biology of the CVs in *Chlamydomonas reinhardtii*. An insertional mutagenesis approach was used to identify proteins involved in osmoregulation. In addition the role of aquaporins in the CV function was investigated using GFP-fusion proteins. We will present new results regarding the regulation and cell biology of the CV in *Chlamydomonas reinhardtii*.

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CHROMERA VELIA – APICOMPLEXAN ALGA WITH PECULIAR PHOTOSYNTHESIS

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We describe photosynthesis, its regulation and capacity for photoacclimation in *Chromera velia*, a recently discovered alga belonging to the group of mostly parasitic apicomplexans. Although the alga has been isolated from coral tissues, it probably does not form standard symbiosis like dinoflagellates, but lives mixotrophically on the surface of the corals. *Chromera* has simple pigment composition. The only chlorophyll present is chl *a*. Interestingly, it is synthesized like in heterotrophs from glycine using the Shemin pathway. Dominant light-harvesting carotenoids are isofucoxanthin and violaxanthin, the latter is also involved in highly effective photoprotective energy dissipation pathway. *Chromera* is the only known alga where the PsaA protein from the reaction center of Photosystem I has two subunits coded on a linear plastid. When grown under constant high irradiance, *Chromera* becomes chronically photoinhibited. However, no photoinhibition is detected when similar light dose is provided under sinusoidal light regime simulating natural irradiance dynamics. The sinusoidal light regime induces strong uncoupling between the net oxygen evolution and carbon fixation that is not observed when cells are grown under constant irradiances. The uncoupling results in low values of the photosynthetic quotient measured during the

photoperiod (PQ 0.7 at noon). We hypothesize that this uncoupling is caused by pronounced oxygenase activity of the type II Rubisco present in *Chromera* and the associated excretion of carbohydrates during photorespiration. In addition, *Chromera* is also subject to strong chromatic adaptation that modulates the composition of its isofucoxanthin containing light harvesting complexes, stoichiometry of photosystems and composition of membrane lipids. We show how the photoacclimation in *Chromera* affects different estimates of the primary productivity that are based either on the variable fluorescence parameters, oxygen evolution or carbon fixation.

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SYSTEMATIC INVESTIGATION OF THE RED ALGAL ORDER HALYMENIALES (FLORIDEOPHYCEAE, RHODOPHYTA) IN AUSTRALIA

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Current taxonomic perspectives regarding the Australian benthic marine red algal flora suffer from a residual bias towards European species, as European references were used to identify most of the flora in the early days of Australian marine Botany. One substantial order of red algae, with an almost ubiquitous presence on Australian coasts, is the Halymeniales. Using the DNA barcode, molecular phylogenetics and traditional alpha taxonomy, the species diversity of this order is being investigated. Cluster analysis of barcode data indicates that our collections have already expanded the order past the known species diversity as represented in the literature. Molecular and morphological data indicate that both completely novel and overlooked species are contributing to this spike in observed biodiversity. Novel diversity has been found within the genus *Tsengia*, as well as for a species, '*Verona peltiformis*', of a putatively novel Tasmanian genus. Cryptic diversity is prevalent in the morphologically diverse genera *Cryptonemia*, *Grateloupia* and *Halymenia*, while *Carpopeltis*, *Gelinaria* and *Polyopes* display some cryptic members. The work of accurately describing the morphology of this flora will be important for revising and understanding the unique biodiversity of the region. A further finding of our

study is that species assigned to the genera *Cryptonemia*, *Grateloupia* and *Halymenia* in Australia do not form monophyletic groups and will require taxonomic revision. Phylogenetic placement of Australian members of the genera *Aeodes* and *Pachymenia* indicates taxonomic clarification is required; similarly, the only Australian *Grateloupia*, *G. ovata*, resolves outside of that genus and requires revision.

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THE DEVONIAN CUTINIZED THALLOIDS ORESTOVIACEAE AS A POSSIBLE LINK BETWEEN HIGHER ALGAE AND INCIPIENT TERRESTRIAL FORMS

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The thickly cutinized thalloid plants of the *Orestovia* group are an archaic-looking, perhaps a 'Lazarus' group of Devonian vegetation. They are widespread in the Middle Devonian of northern Laurasia, forming extensive cuticular mats and coal seams. The cruciate spore tetrads found in the mats and sometimes abundantly preserved on the cutinized thalloids are similar to the Silurian tetrads supposedly produced by incipient higher plants. On account of these findings, the previously postulated liverwort affinity of the tetrad producers seems unlikely. Vascular strands and stomata have been described in the typical *Orestovia*, whereas the morphologically similar forms with gametangial conceptacles lacking genuine stomata may represent aquatic gametophytes. Hypothetically, the origin of isomorphic alternation cycle might have been related to amphibian life style in incipient higher plants, with sexual and asexual generations playing different roles in colonization of terrestrial habitats. Constant occurrence of fungal fruiting bodies in the conceptacles of orestoviacean plants may suggest symbiotic relationships like in extant brown algae and the ascomycete *Mycosphaerella*.

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TO BE THE SAME OR MAYBE CLOSE: GENETIC DIVERSITY IN AN *EMILIANIA HUXLEYI* BLOOM AND IMPLICATIONS FOR PHYTOPLANKTON POPULATION GENETICS

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Coccolithophores, haptophyte microalgae covered with small calcareous scales that contribute ~30% of pelagic carbonates, are central to the process of carbon cycling between the different compartments of the biosphere. The application of either microsatellites or single genes have previously been undertaken to genotype clonal isolates of *Emiliania huxleyi*, a dominant coccolithophore in modern oceans, from different oceanic basins, years and bloom events. Five of the ten previously published microsatellite loci were validated for single-locus genetic determinism and repeatability under asexual reproduction. With these markers and the coccolithophore morphology motif (CMM) genetic marker, a total of 109 clonal isolates collected from across the world, including 49 from a single North Sea bloom event, were genotyped. There were 32 different multilocus genotypes (MLGs, or 63.5%) from the bloom event. Despite the small sample size, there were many more repeated genotypes than previously reported for other bloom-forming phytoplankton species, including a previously genotyped *E. huxleyi* bloom. This study provides new insights into the genetic structure within a bloom event as well as demonstrating the necessary precautions for the interpretation of phytoplankton genetic diversity.

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WHO BENEFITS FROM OCEAN ACIDIFICATION? PREDICTION OF ACIDIFICATION AND EUTROPHICATION EFFECTS ON PRODUCTIVITY OF MACROALGAE

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The negative effects of changing ocean chemistry are

documented for many calcareous species. Here, we estimate the relative effects of ocean acidification (OA) and eutrophication on noncalcifying macroalgae, based on the mechanisms of inorganic nutrient uptake and available data for photosynthetic and/ or growth rate effects of changing dissolved inorganic carbon concentration [DIC]. Our analysis predicts 2 groups of seaweeds that are likely to have enhanced productivity under ocean acidification. (1) The nonbicarbonate using species, having no mechanism of active uptake of dissolved inorganic carbon. Those species were predicted to have increased productivity via direct alleviation of inorganic carbon limitation. (2) Those species with inducible inorganic carbon concentrating mechanisms are predicted to have enhanced productivity under OA via an indirect energetic benefit of elevated [DIC]. Eutrophication in acidified coastal habitats will alleviate nitrogen-limited growth that could result from enhanced production caused by the added carbon availability. Acidification of inorganic nitrogen sources, may additionally reduce energetic cost of nitrogen uptake. Our analysis predicts changes in physiological performances of macroalgae that may affect their demography, interspecific interactions and the composition of ecosystems.

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SAILING IN DEEP WATERS: ALGAL PHYTOCHROMES INFORM OUR UNDERSTANDING OF PHYTOCHROME EVOLUTION AND OF PHOTORECEPTOR ADAPTATION FOR MARINE ENVIRONMENTS

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A single primary endosymbiosis of a cyanobacterium gave rise to the three lineages of the Archaeplastidae: glaucophytes (such as *Cyanophora paradoxa*), rhodophytes (red algae), and Viridiplantae (including land plants). Phytochromes are critical regulators of shade avoidance, photomorphogenesis, and other responses in land plants. Bioinformatics techniques implicate a fusion between a cyanobacterial phytochrome photosensory core (5' end of the gene)

and a pre-existing host histidine kinase (3' end), giving rise to plant phytochromes. Both plant and cyanobacterial phytochromes exhibit photoconversion between red- and far-red-absorbing forms; this red/far-red photocycle is also found in phytochromes from desmid algae. Similarly derived phytochromes are present in *C. paradoxa*, implicating early evolution of plant phytochrome in Archaeplastidae. Phytochrome has been lost in extant rhodophyte genomes and in algal members of the Viridiplantae such as *Chlamydomonas*, *Volvox*, *Chlorella*, *Bathycoccus*, and *Ostreococcus*. Peculiarly, phytochromes are found in cryptophyte algae, which acquired photosynthesis via secondary endosymbiosis of a rhodophyte. We have now characterized phytochromes from the cryptophyte *Guillardia theta* and the green algae *Micromonas* and *Nephroselmis*. Strikingly, all three photosensors do not exhibit the classic red/far-red photocycle. Instead, these newly identified phytochromes all exhibit an orange/far-red photocycle that we hypothesize is an adaptation for the marine environment. We therefore expect that such an adaptation will also have arisen in brown algal phytochromes, which have independently evolved in the marine environment.

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PHYLOGENY OF THE BATRACHOSPERMALES (FLORIDEOPHYCEAE, RHODOPHYTA)

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A multigene phylogeny of the strictly freshwater order Batrachospermales was inferred via maximum likelihood. The phylogeny was based on a nucleotide dataset comprised of five plastid genes, two mitochondrial genes, and two nuclear genes. The taxon-rich phylogeny included all major groups within the Batrachospermales and represents the most inclusive analysis for this order to date. Nearly all batrachospermalean taxa were placed into two well-supported clades. One clade was composed of the sections *Batrachospermum*, *Helminthoidea*, and *Setacea*. Additional taxa in this clade included the "Australasian Group", *Lemanea*, *Paralemanea*, *Petrohua*, *Sirodotia*, and *Tuomeya*. The second clade was comprised of the sections *Acarposporophytum*, *Aristata*, *Macrospora*, *Turfosa*, and *Virescentia*. The

genera *Kumanoa* and *Psilosiphon* were placed in the second clade as well. A single species, *Balliopsis prieurii*, could not be placed into either of the aforementioned clades based on the inferred phylogeny. Currently, all taxa have been placed into a single family, the Batrachospermaceae, however our results suggest the erection of two new families for the Batrachospermales based on the aforementioned clades.

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FACTORS CONTROLLING SPATIAL AND TEMPORAL DYNAMICS OF DRIFTING MACROALGAE IN PATOS LAGOON ESTUARY (RS, BRAZIL)

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In the Patos Lagoon estuary (PLE), temporal changes in the structure of benthic primary macroproducers have been observed, with phases dominated by opportunistic green macroalgae. This study investigates the variability of macroalgae occurrence in shallow areas of PLE and how hydrological and meteorological factors affect their distribution and abundance. The results of monthly monitoring show a very high variability in the macroalgae abundance composed mainly of drifting mats of *Ulva* and *Cladophora* spp. Peaks of biomass and coverage were observed in early summer and autumn during periods of high water temperature and low turbidity. Analysis of spatial distribution showed that the highest biomass values were associated to sites where inorganic nutrients in the water and sediment organic matter were higher. In addition, differences in drifting mats coverage among sites were related to their exposure to winds and waves (Wave Exposure Index), which causes biomass accumulation in shallow subtidal and intertidal areas. Further analysis of nitrogen and carbon contents in algal tissue and sediments will be conducted to elucidate the role of sediments as nutrient source and sink controlling algal-dominated phase states.

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EUTROPHICATION AND MACROALGAL BLOOMS IN COASTAL WATERS OF

SOUTH FLORIDA: TAXONOMIC COMPOSITION AND N:P RATIOS

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Coastal waters of South Florida have experienced unprecedented blooms of benthic macroalgae with increasing eutrophication during the past several decades. In coral reef environments off Monroe, Dade and Broward counties in South Florida, blooms of the phaeophytes *Dictyota* spp., the calcareous chlorophytes *Halimeda* spp., and the cyanobacterium *Lyngbya* have developed. On more northern coral reefs off Palm Beach County, extensive blooms of the chlorophytes *Codium isthmocladum* and *Caulerpa* spp. have recurred since 1990. In Lee County off Florida's southwest coast, massive blooms of the drift rhodophytes *Gracilaria*, *Hypnea*, and *Agardhiella* emerged in 2003. Measurements of dissolved nitrogen and phosphorus in the water column and elemental C:N:P ratios in algal tissues indicated that the spatial taxonomic shifts in these macroalgal blooms was related to N:P ratios. Relatively high N:P ratios (35:1-70:1) occurred in Monroe, Dade and Broward counties, compared to lower values in Palm Beach County (33:1) and Lee County (20:1). These differences in N:P ratios and N- vs. P-limitation result from variation in geological substrata (carbonate-rich vs. siliciclastic) as well as anthropogenic nutrient enrichment from the watersheds.

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ASSESSING THE RELATIONSHIPS BETWEEN ELECTRON TRANSFER AND CARBON FIXATION IN NITROGEN LIMITED MARINE PHYTOPLANKTON

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Primary productivity is essential to describing structure and function of aquatic ecosystems. Development of bio-optical sensors, such as fast repetition rate (FRR) fluorimeters, have opened up

new avenues for deriving proxies of in situ primary productivity from measurements of electron transfer rates (ETR). The approach, however, suffers from the limitation that FRR fluorimeters determine productivity in a photosynthetic 'currency' of electrons produced by the splitting of water at PSII whereas most aquatic disciplines are interested in carbon-specific rates of primary productivity. Thus, ETR must be converted to carbon-specific primary productivity rates via a conversion factor, that is the ratio of ETR:carbon fixation (units: mol electrons/mol carbon fixed). This variable, however, is dependent upon the physiological state of phytoplankton. Here, we present results of a comprehensive laboratory study examining the effect of nitrogen availability on the variability of the conversion factor in model phytoplankton species from coastal and open ocean waters. Our results show a strong uncoupling of electron transfer and carbon fixation leading to large intraspecific and interspecific variability in the relationships between these two variables.

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OBSERVATIONS ON THE TAXONOMY OF CRYPTOPHYTES OF THE TELEAULAX-COMPLEX

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Teleaulax and *Plagioselmis* species are among the most common marine cryptophytes. They are regarded as non-monophyletic genera and, together with the monospecific *Geminigera*, form a natural group characterized by a thylakoid disposition different from other cryptophytes. To gain insight into the taxonomy of this genera-complex, new isolates are being studied, as a result of which two new species, *T. gracilis* and *T. minuta*, were recently described. Due to the apparent non-monophyletic nature of the genera, observations on the type species are especially important. Strains consistent with Butcher's description of *Cryptomonas acuta*, the basionym of the type species of *Teleaulax*, are suggested to be also consistent with *T. amphioxeia* sensu Hill, and different from *T. acuta* sensu Hill.

THE STUDY OF TROPICAL PLANKTON APPEARANCE IN EAST SEA, KOREA

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Global warming has resulted in lots of environmental changes. This study was conducted to determine the biological adjustment to changes in the ocean ecosystem such as new tropic plankton appearances in a temperate climate area. Therefore, in this study, we focused on appearance of tropical plankton and the extent of adaptations for increases in water temperature. We conducted sampling at 16 stations from 28 May to 3 June and 35 stations from 5 August to 10 August 2012. Several tropic species were found near the Dokdo, which is located in the eastern part of Korea. Their presence near the Dokdo is probably related to the Kuroshio Current, which affected their influence from spring to the summer in East Sea. The 5 species, *Cochlodinium* sp., *Dinophysis caudata*, *Gonyaulax spinifera*, *Ostreopsis* sp., *Phalacroma mitra* were observed at 9 of the 16 stations. The 4 species other than *Cochlodinium* sp. were observed at 7 of the 35 stations. 8 genera were observed during May but only 4 genera were observed; most of the observed species in August were diatoms. When we compared biomass based on the cell counting, the numbers were similar in spring (30.6~252.6 cells L⁻¹) and summer (35.0~260.0 cells L⁻¹) in 2012. The dominant species were *Chaetoceros curvisetus*, *Chroomonas* sp., *Corymbellus aureus*, *Leptocylindrus minimus*, *Proboscia alata*, and *Prorocentrum balticum* in spring and *Chaetoceros affinis*, *Ch. curvisetus*, *Gymnodinium* sp., *Leptocylindrus minimus*, *Nitzschia* sp., *Rhodomonas* sp., *Thalassiosira* sp. in summer. Based on this study, even though, the water temperatures were higher in summer than in spring in this general temperate climate area, a similar number of tropical species were observed spring season. It could be the explanations of the seasonal effect of Kuroshio Current in East Sea, Korea.

CHARACTERIZATION OF PHOTORECEPTORS AND THEIR MACHINERIES INVOLVED IN TWO

TRACK PHOTOMOVEMENT OF SPIROGYRA VARIANS

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The filamentous green alga *Spirogyra varians* shows two track photomovement; a positive phototropic movement to blue light and an irregular twisting movement to red light. The reversal of red light movement by a short exposure (<30s) to far-red light implied that phytochrome is involved in photomovement too. Two phototropin genes and three phytochrome isoforms were isolated and characterized for the first time in *Spirogyra*. Both photoreceptor genes showed high homology with land plants but each isoforms of phototropin and phytochrome contained some unique structures. Real time PCR analysis showed that the expression level of each isoform was differently affected by the prolonged exposure of filaments to blue and red light. Previous studies showed that the blue and red light responsive movements are controlled through different signaling pathways. Seven different GTPase genes were isolated and the expression level was compared before and after exposure to red and blue light. Among them, the Rop (Rho small GTPase of plants) family of small GTPase genes showed dramatic response to change of light condition. The potential molecular mechanisms of Rop GTPase signaling involved in cell motility were discussed together with the role of actin and tubulin as machineries.

THE GEOGRAPHIC SCALE OF SPECIATION IN THE MARINE RED ALGA PORTIERIA

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The role of geographic isolation in marine speciation has been contested because of the high dispersal potential of marine species and the apparent lack of dispersal barriers in the sea. Compared to marine invertebrates and fish, most seaweed species are considered poor dispersers. This limited dispersal capacity may reflect on diversity patterns and the

spatial scale at which speciation takes place. We assessed species diversity, distribution patterns and phylogenetic relationships within the marine red algal genus *Portieria* based on multilocus data and analyses combining phylogenetic and population genetic principles. In stark contrast with the current morphology-based assumption that the genus includes about seven species, including one widely distributed species in the Indo-West Pacific (*P. hornemannii*), DNA-based species delimitation resulted in the recognition of more than 90 species. Species distributions were found to be highly structured with most species having narrow ranges. Our results indicate that speciation in the marine environment may act at much smaller geographical scales than is commonly assumed. The most likely explanation to account for the observed diversity is a peripatric model of speciation, involving founder events resulting from long distance dispersal and subsequent differentiation of the established populations. Temporal analyses of diversification indicate a gradual pattern since the Eocene, and long term persistence of ancient lineages within confined regions of the Indo-Pacific. The Indo Malay archipelago (IMA or Coral Triangle) was found to be particularly diverse. Our phylogenetic data indicate that this diversity resulted from a combination of species accumulation via island integration through tectonic movement, in combination with speciation within the IMA as a result of increased geographical complexity of the region from the Miocene to Pleistocene.

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BIOINDICATORS OF MARINE ENVIRONMENTS: USING SEAWEEDS AS A TOOL FOR BIOMONITORING THE QUALITY OF COASTAL WATERS RESPONSE TO A PROBLEM OF MASSIVE SEAWEED BEACHING

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In the context of massive seaweed beaching during summer along the French coast of the English Channel in the region of Calvados, the aim of this study was to use the seaweeds to understand the environmental conditions responsible for this phenomenon. The evaluation of eutrophication of coastal waters is based

on the use of delta-¹⁵N signatures in seaweed tissues to identify the sources of nitrogen. Five naturally occurring seaweeds (*Ulva sp.*, *Fucus vesiculosus*, *F. serratus*, *Chondrus crispus* and *Porphyra sp.*) were collected at two sites (Baie des Veys and Courseulles-sur-Mer) at two-monthly intervals in 2012. Isotopic signatures ranged from 5.79 ± 1.13‰ to 14.16 ± 0.38‰ at the 17 sampling points distributed between the two sites. Seasonal variations in isotopic signatures were observed with low delta-¹⁵N values in spring (5.79 ± 1.13‰ to 9.37 ± 0.39‰) and high delta-¹⁵N values in summer, autumn and winter (8.18 ± 0.19‰ to 14.16 ± 0.38‰). Differences in delta-¹⁵N signatures were detected between sampling points and in the different species of seaweeds.

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EXTENSIVE CHLOROPLAST GENOME REARRANGEMENTS AMONG SPECIES OF THE GENUS *ELLIPTOCHLORIS* (TREBOUXIOPHYCEAE, CHLOROPHYTA)

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Chloroplast genomes of green algae are highly variable in architecture within and across major taxonomic groups. To investigate the evolution of chloroplast genomes at a shallow phylogenetic depth, we used a nextgen approach to sequence the chloroplast genomes of *Elliptochloris marina* and *Elliptochloris reniformis*. The two genomes vary in size, the *E. reniformis* genome is 110 kb and *E. marina* is 145 kb. Extensive chloroplast gene rearrangements are found between the two species. In contrast, these taxa have very low pairwise sequence divergence in orthologous genes. Like the majority of published trebouxiophyte chloroplast genomes, these species have no inverted repeats. They also share similarities with the published genome of *Coccomyxa subellipsoidea*, having large spacer regions, and a high GC-bias. Pairwise sequence divergence of *Coccomyxa* and the two *Elliptochloris* species is twice as great as that found between the *Elliptochloris* species. However, the amount of chloroplast genome rearrangement is relatively consistent among all three species. This study demonstrates extensive chloroplast architectural rearrangements among congeneric Chlorophyta, while also illustrating of some shared

genome features among this closely related group of green algae.

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NATURAL HISTORY OF THE GREEN ALGAE-SALAMANDER SYMBIOSIS

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Green algae form symbioses with diverse organisms, including fungi, ciliates, embryophytes, invertebrates, and vertebrates. *Oophila amblystomatis* was described in 1909 for green algae that colonize salamander eggs. Recent studies indicate an intimate cellular association between the algae and salamander embryos. An important step in understanding the natural history of this symbiosis is to assess the level of variation found in the algal symbionts, and the possible source(s) of the algae. We tested the hypothesis of monophyly of salamander algae at different hierarchical levels, from among eggs of a single clutch, clutches within ponds, ponds from different geographic regions, and between salamander species. Phylogenetic analysis of rbcL sequence data from algae isolated from multiple eggs of one clutch show monophyly and near sequence identity. However, algae associated with eggs at different spatial scales show variation and form distinct lineages in Volvocales, Chlorophyceae. Our analysis also indicates that algae grown from spermatophores deposited in lakes by adult males are similar to those found within eggs. Our results raise intriguing questions regarding the specificity and evolution of the algal-salamander symbiosis.

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BAYES FACTOR FOR DATA COMBINABILITY: APPLICATION TO THE PHYLOGENY OF SPHAEROPLEALES (CHLOROPHYCEAE, CHLOROPHYTA)

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Sequencing nuclear and plastid genes from taxa in the order Sphaeropleales as part of the GrAToL project

has yielded surprising amounts of incongruence among gene trees even in the same genomic compartment. For example, no single tree topology was sampled for any 2 of 7 genes in independent Bayesian analyses despite considerable sharing of particular clades. Bayesian concordance analysis (BCA) was constrained in this case to conclude, with certainty, that the minimum number of topologies is 7 and to return a consensus of the pooled trees from all 7 analyses as the concordance tree. Such an extreme position appears to result from not considering data from more than one gene simultaneously. Recent improvements in marginal likelihood estimation now make it possible to estimate a Bayes Factor (BF) for combinability, which uses posterior samples from a combined analysis in addition to separate single-gene analyses to determine the odds in favor of combinability. In contrast to the BCA results, we show that leaving out just 1 of 7 genes is enough to tilt the odds in favor of combinability.

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DETECTING SALINITY ECOTYPES IN GAMETOGENESIS IN THE KELP *ALARIA MARGINATA*

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Reducing salinity has been found to increase gametogenesis in some species of kelps. The optimal salinities for both oogenesis and spermatogenesis were determined for gametophytes of three morphospecies of *A. marginata*. Morphospecies *Alaria marginata* and *A. nana* were collected from the west coast of Vancouver Island, British Columbia, Canada, with salinities of 27-30 psu. *Alaria tenuifolia* was collected at the city of Vancouver, with salinities of 24-28 psu. Isolated female and male gametophytes were fragmented and cultured at a controlled density in seawater medium with salinity ranging from 12 to 33 psu. Eggs were counted after 14 days. Sperm were released after 14 days using medium containing the pheromone lamoxirene from fertile female gametophytes, and counted. Oogenesis was highest at 27 psu for *A. marginata* and 18-21 psu for *A. tenuifolia*. Spermatogenesis was highest at 21-27 psu for *A. nana* and 15-21 psu for *A. tenuifolia*. The optimal salinity for gametogenesis in these

morphospecies reflects differences in salinity in the habitats from which they were collected, indicating ecotypic differentiation in regards to salinity.

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***EUSTIGMATOS* CF. *POLYPHEM* (EUSTIGMATOPHYCEAE), A POTENTIAL MICROALGA FOR BIODIESEL PRODUCTION**

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Eustigmatos cf. polyphem is a yellow-green spherical unicellular soil microalga belonging to Eustigmatophyceae. The maximum biomass concentration obtained in batch cultures with modified BG-11 medium reached 7.91 g L⁻¹. This microalga could accumulate massive storage lipids. The total lipid content was up to 62% of dry biomass under low concentration of nitrogen supply. The major component of total lipids was neutral lipid, namely, triacylglycerol (TAG). The major fatty acid profiles of *E. cf. polyphem* were palmitic acid (C16:0), palmitoleic acid (C16:1), oleic acid (C18:1), and eicosapentaenoic acid (C20:5); the sum contents of these four fatty acids in biomass attained 81.50% of total fatty acids (TFA). The main pigment compositions of *E. cf. polyphem* were composed of chlorophyll *a*, violaxanthin, vaucherixanthin, zeaxanthin and β-carotene. Our studies demonstrate that *E. cf. polyphem* could be a promising oil-producer for commercial biodiesel production due to its fast growth, high lipids productivity and defined fatty acid profiles.

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MORPHOLOGICAL STUDIES OF *FALLACIA* SPECIES IN JAPAN

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Eight *Fallacia* species including *F. tenera*, *F. litoricola*, *F. fracta*, *F. hodgeana* comb. nov. and four unidentified species collected from intertidal area and river mouth in Japan were observed in detail. These species share common characteristics such as one

basically H-shaped plastid, lateral sterna, finely porous conopeum, external terminal fissures curved or hooked in the same direction, small helictoglossae located at two distal ends of raphe internally, etc. The pattern of the canal system (lumen between primary silica layer and conopeum) in *Fallacia* generally resembles that in *Diploneis*. The depressed lateral sterna and conopeum compose a longitudinal or lyre-shaped canal with pore or slit openings on both sides of terminal fissures. The uniseriate stria with lateral virgae and conopeum compose an elongated chamber, which open to canal at proximal terminal. The canal system open to outside via the openings and fine pores of conopeum. The protoplasm could communicate with exterior via the canal system or a number of areolae on the mantle of valve in some species. The cingulum is composed of two bands, a valvocopula and a thin pleura, in most cases only ligula was observed.

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PHYLOGEOGRAPHY OF SPECIES IN THE *MAZZAELLA OREGONA* CLADE (GIGARTINACEAE, RHODOPHYTA) IN THE NORTHEAST PACIFIC

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We examined genetic variation among isolates of species in the *Mazzaella oregona* clade: *M. oregona*, *M. parksii*, *M. phyllocarpa*, and *Mazzaella* sp. (this species has been described, but a new combination has yet to be made in *Mazzaella*) using the nuclear ribosomal ITS region, the chloroplast *rbcL* gene, and the mitochondrial COX1 “barcoding” gene. Based on our own and published results, *Mazzaella oregona* is distributed from southern California to Kodiak Island, Alaska. There is little genetic variation in populations from California to Alaska except for a distinctive genotype found in individuals from the eastern Strait of Juan de Fuca in southern British Columbia and northern Washington and near Prince Rupert in northern BC. *Mazzaella parksii* is distributed from Mendocino Co., CA, north and westward to at least the westernmost Aleutian Island, Attu, Alaska. Populations show significant differentiation between southeastern populations (from southern Oregon to the Kodiak archipelago, Alaska) and northwestern

populations (from the Kodiak archipelago to Attu Island, Alaska). *Mazzaella phyllocarpa* is recorded from Russia to Southeast Alaska and north to St. Lawrence Island in the northern Bering Sea. Although there is genetic variation among specimens from Alaska, there is no geographic pattern to this variation. *Mazzaella* sp. is distributed from the Commander Islands, Russia, to northern Vancouver Island, British Columbia. Although there is variation among individuals, there is little geographic pattern to this variation. These results resemble those found for other intertidal marine organisms in the northeast Pacific in that the high intertidal species showed the most phylogeographic differentiation, and a biogeographic break appears in the northwestern Gulf of Alaska for at least one of the species.

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EFFECTS OF CLIMATE CHANGE AND NUTRIENT LOADING ON PLANKTON BLOOMS AND TROPHIC ORGANIZATION IN PERDIDO BAY (NORTHEASTERN GULF OF MEXICO)

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In the Perdido system, high nutrient concentrations from pulp mill effluents during drought conditions stimulated extensive blue-green (*Merismopedia tenuissima*) algae blooms in a receiving creek whereas the combination of high nutrient concentrations and high nutrient loading during episodic high flow conditions led to sustained blooms of damaging raphidophyte (*Heterosigma akashiwo*) populations in the bay. Climatological conditions along with ecological characteristics of individual plankton species affected bloom initiation. The response of food webs to blooms should be placed within the context of habitat distribution relative to the point of loading, species-specific bloom composition, and temporal aspects of drought occurrence. The resilience of the bay relative to the repetitive nature of the plankton blooms and the bloom sequences is dependent on complex temporal changes (natural and anthropogenic) that occurred in both the creek and the bay over interannual periods. Repetitive blooms had cumulative, adverse impacts on food web organization through time in both the creek and the bay. Such

occurrences should be placed within the context of long-term drought/flood conditions that have changed in the eastern Gulf over the past 30 years.

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OBSERVATIONS ON THE ABUNDANCE AND DISTRIBUTION OF THE KELP *SACCHARINA GROENLANDICA* IN THE ATLANTIC OCEAN, EMPHASIZING NORTH AMERICA

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Until recently, two members of the Laminariaceae were reported from the Maritime Provinces of Canada (*Saccharina latissima* and *Laminaria digitata*); however, a third species, *S. groenlandica*, was revealed using molecular techniques. In the Pacific Ocean *S. groenlandica* displays either the non-digitate (*S. latissima*-like) or digitate (*L. digitata*-like) morphology. As a result of its likeness to both of the Atlantic species, *S. groenlandica* has gone undetected and has been incorrectly lumped with either *S. latissima* and/or *L. digitata* in previous studies. Our objective was to determine the relative abundance of *S. groenlandica* and whether it expresses the non-digitate and digitate phenotypes across a wave exposure gradient in the Maritime Provinces as observed in Pacific populations. We found that digitate *S. groenlandica* comprised ~1/4 of all individuals sampled and is more abundant than *L. digitata*. We are currently working to determine the seasonality and phenology of all three kelp species as previous reports, especially for *L. digitata*, will be based on data from more than one species. We are also determining the geographic range of *S. groenlandica* in the North Atlantic Ocean.

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FIRST ASSESSMENT OF THE RELATIVE ABUNDANCE OF MAERL-FORMING CORALLINE ALGAE WITH DNA BARCODES

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Under the assumption that they are major components of maerl beds in Europe, two non-articulated coralline red algae (*Lithothamnion corallioides* and *Phymatolithon calcareum*) are partially protected by the EU Habitats Directive. However, most of the current knowledge on the composition of maerl beds relies on a morphology-based species recognition that is challenging, to say the least. Now, this state of affairs can be improved thanks to new alternatives that, unlike morphology, allow the unambiguous assignment of each specimen to a particular species. Here, we report the first DNA barcoding assessment of the relative abundance of maerl-forming algae. The *psbA* gene was sequenced for 1076 specimens from 16 locations scattered along 1800 km from Ireland to South Portugal. At each location, specimens were randomly collected along a linear transect to obtain quantitatively comparable estimates. Three major maerl-forming species, rather than two, comprised 97% of the collections. The traditionally recognized *L. corallioides* and *P. calcareum* dominated in the British Isles and French Brittany, but they were gradually replaced by *P. purpureum* in Northwest Spain and South Portugal.

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THE OCCURRENCE OF *DICTYOTA CANARIENSIS* (DICTYOTALES) IN THE TROPICAL REGION FROM GULF OF MEXICO

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The brown alga *Dictyota canariensis* (Grunow) Tronholm has been reported from the European Atlantic region. In the present work the authors record for the first time the presence of this species in Mexican waters, at Montepio, Veracruz. The specimens were collected in December 2011, growing epilithically in the intertidal zone on moderately exposed rocky shores. We carried out phylogenetic analysis, combining the partial sequences of *psbA* and *cox1* genes of 46 taxa. The low uncorrected “p” distance values obtained for each gene (0.3% for *psbA* and 1.8% for *Cox1*) showed that it belong to the

taxonomic entity. The morphological results showed that the Mexican specimen share morphological characteristics with *D. canariensis* like the presence of square-like pigmented patches near the apices, margins with triangle-shaped teeth, rounded to obtuse apices and a unilayered medulla. The present study expands the geographical distribution of *D. canariensis* to the tropical region from the Gulf of Mexico in the Western Atlantic Ocean.

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GENOMIC INSIGHTS IN PROCESSES UNDERPINNING THE INFECTION OF *ALEXANDRIUM TAMARENSE* BY THE PARASITE *AMOEBOPHRYA* SP.

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The toxigenic dinoflagellate *Alexandrium tamarense* is one of the most prominent microalgae known to form harmful algal blooms. Its parasitic pathogen, the dinoflagellate parasitoid *Amoebophrya* spp. has been observed to infect and dominate the blooms, but the genetic background of the host-parasite interaction and its ecological consequences remain elusive. The objective of this study was to obtain genomic insights into the process of infection between the parasite *Amoebophrya* and the host *A. tamarense*. We identified 14,455 genes, which were predicted by an extensive EST dataset (>900,000 reads/313MBp) generated over the infection cycle. The functional annotations of 2,938 genes were based on NCBI Nr, Swissprot, Pfam, KOG/COG classification and were finally manually curated. The infection cycle analyses at three time points showed that the free-swimming dinospores (0 hour) had evolved an extensive gene set to survive, whereas intensive signal transduction and senescence-associated protein were observed during parasite penetration (6 hours), and at final infection stage (96 hours) cell division and proliferation were high expressed. We present an initial analysis of this data resource which already yielded new insights into the complex host-parasite interaction.

DNA BARCODING AND GEOGRAPHIC RANGE OF MACROALGAE FROM CENTRAL-SOUTHERN CHILE

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Field trips carried out in rocky intertidal and shallow subtidal along the Chilean coast from 33°1' S to 43°54' S between 2011-2013 allowed us to evaluate the distribution range of several macroalgal species (Rhodophyta and Phaeophyceae). Sampling also provided material to carry out DNA barcoding analysis (mitochondrial Cytochrome Oxidase I gene - COI) in order to test the effectiveness of this method. With the data already obtained, we provided information of algal biodiversity from locations with restricted access and scarce information (e.g. southern Chiloé Island, Melinka Island). Six red algal species extended their distribution range and from those, four corresponded to non-indigenous species. Some species increased their range up to 1000 km. Mitochondrial COI gene sequences data provided rapid and accurate approach for species-level identification in several red and brown macroalgae specimens collected and will be valuable information for taxonomical and phylogeographical studies along the Southeastern Pacific. This study was funded by CONICYT-Chile through the FONDECYT 11110437 project.

DIVERSITY OF THE LAURENCIA COMPLEX (CERAMIALES, RHODOPHYTA) IN LUSITANIAN MACRONESIA

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Our aim was to investigate the diversity of the *Laurencia* complex in the Azores, Madeira, Salvagens

and Canary Islands, Atlantic oceanic archipelagos, which belong to the same ecoregion, Lusitanian province of the Macaronesian region. We assessed the species limits among members of the complex using DNA barcode data (partial sequences of mitochondrial COI and nuclear LSU markers). The information obtained from the analyses of COI and LSU sequences was consistent; both markers displayed adequate signal for the delineation of species. In addition, preliminary phylogenetic analyses were completed using three independent markers (*rbcL*, LSU, and COI) as well as the combined data set, in the aim to infer the phylogenetic relationships and biogeographic affinities of members of the complex from Macaronesia. Our results revealed the existence of several putative new species and a potential new genus included in the *Laurencia* complex. Prior the proposition of new taxa, more thorough morphological and molecular analyses of specimens of type localities, together with the results obtained in our study, are essential to assign the available taxonomic name to the different species uncovered in our studies.

DIVERSITY OF FRESHWATER ALGAE OF WEST BENGAL, TAXONOMIC ASPECTS AND POTENTIAL USES

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The state of West Bengal lies in the eastern part of India. The state is mainly a riverine plain with its northern fringes in the foothills of the Himalayas and the Bay of Bengal on the south. The present study delves into the taxonomically diverse algal groups and their beneficial aspect in human life. Some algal genera of Desmidiaceae namely *Actinotaenium*, *Brachythecca*, *Bourrellyodesmus*, *Spinocosmarium* and *Haplotaenium* are recorded for the first time from Bengal and also to the Indian subcontinent. Under these genera several new taxa have been introduced as new to science. *Brachythecca* is rare and known from two countries only [Papua New Guinea (Gontcharov & Watanabe 1999) & New Zealand (Fumanti & Alfinito 2004)]. This finding is significant for taxonomical, economical and also ecological aspect. Microalgae have a significant attraction as natural source of bioactive molecules which are difficult to produce by

chemical synthesis. With increasing urbanization and the subsequent loss of freshwater bodies, a detailed enumeration and characterization of this algal diversity is a necessity before many of these algal species disappear forever.

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HOW THE DIATOM GOT ITS SHAPE

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The diversity of shapes that diatom valves exhibit is well known, ranging from circular or elliptical through triangles, squares and polygons, to elongate spindle- or needle-like forms, which may be straight or bent. What is much less well known are the processes by which these shapes are generated during the expansion of the special cell (auxospore) that allows diatoms to regenerate large cells after the slow diminution during repeated divisions of normal vegetative cells. Shape develops successively during one or more of three stages: (1) the origin of the auxospore from a zygote, (2) the expansion of the auxospore, and (3) the formation of the initial cell within the auxospore. Changes during (1) are probably controlled by the cytoskeleton, and the cytoskeleton must also be involved in (3), together with anchor points binding the cell to the auxospore wall (e.g. during post-expansion modification of shape in some *Nitzschia* species). The basic principle of shape morphogenesis in stage 2 is the same as in placoderm desmids such as *Micrasterias*: unequal hardening of different regions of the auxospore wall constrains expansion to one, two or several growing tips. In most cases the unequal hardening involves formation of a series of silica bands (the perizonium) at right angles to the expansion axis. Control of the rate of hardening relative to expansion allows diatoms to construct parallel-sided, tapering or (more rarely) widening auxospores. Perizonia occur both in pennate diatoms e.g. *Diploneis*, *Neidium* and *Rhabdonema* and also in elongate or angular centrics, e.g. *Chaetoceros*, *Triceratium*, *Trigonium*. However, similar shapes can be formed with or without a perizonium, since *Hydrosera* constructs its triangular shape without any perizonial bands, its auxospore having instead a felt-like mass of narrow siliceous strips. Where there is no

differential hardening of the auxospore wall, expansion is isometric (e.g. *Actinocyclus*, *Melosira*) and valves are circular.

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COMPARISON OF NUTRIENT REQUIREMENTS OF COMMON DIATOMS FROM FOUR ECOREGIONS IN GEORGIA

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Microbial biodiversity remains largely unknown and constantly altered due to anthropogenic alterations of the environment. The goal of this research is to determine which common species reported from the State in Georgia had low optima for both limiting nutrients and were decreasing with increase in nutrient concentrations in natural stream. Nutrient optima and tolerances were compared within and between land use categories and ecoregions. Assessment of all algal community structure presented the majority of streams were dominated by diatoms. Only 3% of the taxa identified had relative abundance above 10% and appeared in at least 1 site in the 4 ecoregions and used for comparison. The northern ecoregions of Georgia had low diatom species density and were dominated by *Achnanthydium minutissimum*. This taxon had low TP optimum but was able to tolerate higher nitrogen concentrations. *Gomphonema rhombicum* had the lowest TP and TN optima, taxa like *Geissleria aikenensis* had high TP optima but were unable to compete with increase of nitrogen. Piedmont communities were dominated by several *Achnanthydium* species, together with taxa from *Eunotia*, *Gomphonema*, *Frustulia* and *Pinnularia*. Piedmont streams had the highest diatom densities, although total abundances varied across sites, therefore there was no density compensation related to nutrients.

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DIGITAL GENE EXPRESSION PROFILES OF PYROPIA HAITANENSIS RESPONDING TO HYPEROSMOTIC STRESS USING SOLEXA SEQUENCING TECHNOLOGY

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Pyropia haitanensis is an economically important marine crop, worth over US\$100 million annually. As a high intertidal species, its blades survive a loss of up to 90% of cellular water demonstrating its extreme tolerance to desiccation. To define the critical acclimation mechanisms to the desiccation in *P. haitanensis*, we profiled gene expression pattern under three different degrees of water loss plus rehydration, using the Illumina deep sequencing technology. A total of 1,681 genes showed significantly differential expression between the control (0% water loss) and the moderate dehydration (30% water loss), highlighting the key roles of molecular chaperones, induction of apoptosis, MAPK signaling pathways, antioxidant system and ELIP. Only eighteen differential expression genes were found between the moderate and the severe dehydration (80% water loss), indicating their similar expression patterns. Of the 1,199 differentially expressed genes in response to rehydration, many genes were involved in induction of apoptosis, chaperone/cochaperone proteins and antioxidant system. The comprehensive high-resolution analysis of gene expression changes associated with hyperosmotic stress provided key resources for understanding the biology of desiccation tolerance in intertidal red seaweed.

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EVOLUTION OF EUGLENOID CHLOROPLASTS

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Euglenoids are an ancient eukaryotic lineage that may have existed as early as 2 billion years ago. Following the evolution of green algae, some euglenoids acquired chloroplasts through secondary endosymbiosis from a green algal donor. Previous studies have shown great changes in the organization of the chloroplast genome along the photosynthetic lineage. This study compares the chloroplast genomes of *Phacus inflexus* and *Lepocinclis tripteris* to determine how rapidly the chloroplast is evolving in two closely related genera. Analyses show that, despite significant difference in overall genome size, both genomes contain almost all

the same 87 chloroplast genes suggesting a high level of gene conservation. However, pairwise comparison of individual genes show marked differences in nucleotide sequence, individual gene size, intron number and intron position. Additionally, gene mapping shows large rearrangements in which entire blocks of genes change position within the genome or move from one DNA strand to the other.

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MOLECULAR ASPECTS OF CARBON ACQUISITION MECHANISMS IN MARINE DIATOMS

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It is estimated that diatoms are responsible for about one fifth of the total biological CO₂ fixation on the Earth, playing a vital role in global primary production and the global cycle of elements. Many physiological evidences have revealed that marine diatoms take up both CO₂ and HCO₃⁻ from the surrounding seawater. However, little is known about molecular mechanisms of how dissolved inorganic carbon (DIC) is taken up and delivered to ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO). There are two model diatoms so far intensively studied at molecular levels, the pennate *Phaeodactylum tricorutum* and the centric *Thalassiosira pseudonana*. Our recent study has shown that there are numerous mammalian type solute carrier (SLC) transporters in these strains and that these function as major plasmamembrane type HCO₃⁻ transporters. Knowledge on molecular and biochemical bases of factors which may control the flux and state of intracellular carbon were also improved recently. Enzymes which may participate in the biophysical CCM and C₄ type biochemical CCM were localized in these two strains of diatoms. The results clearly showed an absence of known type carboxylation enzymes in the cytosol and decarboxylation enzymes in the stroma in two strains. The localization of carbonic anhydrases were likely diverse significantly between these two strains; i.e., besides the mitochondrial CAs, CAs in *P. tricorutum* were localized in the pyrenoid and chloroplastic envelopes while CAs in *T. pseudonana* are localized at the periplasm and the cytosol but so far not detected in the chloroplastic system. Based upon these evidences,

molecular mechanisms and diversity of carbon acquisition system in marine diatoms will be discussed.

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DELINEATION OF A NEW SNOW ALGAE SPECIES OF *CHLOROMONAS* FROM A JAPANESE MOUNTAINOUS AREA, BASED ON POLYPHASIC METHODS

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The traditionally defined genus *Chloromonas* (pyrenoid-lacking *Chlamydomonas*; Ettl 1970) includes several snow algae species (Ettl 1983, Süßwasserflora). Pröschold et al. (2001, Protist) redefined the genus *Chloromonas* by a monophyletic group (*Chloromonas*-clade) composed of both pyrenoid-lacking and -containing species and light microscopic (LM) morphology. Hoham et al. (2002) resolved a robust clade composed entirely of snow algae species lacking pyrenoid (clade A2) within the genus *Chloromonas*. Recently, Matsuzaki et al. (2012, 2013) delineated five mesophilic species of *Chloromonas* based on comparative morphological analyses, multigene phylogeny and comparison of secondary structures of nuclear ribosomal DNA internal transcribed spacer 2 (ITS2). However, no snow algae species of *Chloromonas* have been classified using such polyphasic methods (Pröschold & Leliaert 2007). Here, we carried out a taxonomic study of a new snow algae strain (GsCl-11) of *Chloromonas* originating from Mt. Gassan in Northern Japan. Under LM, this alga was similar to *C. tughillensis*, a snow alga from North America, in having an ellipsoidal cell shape without a prominent anterior papilla and lacking an eyespot. However, *Chloromonas* sp. GsCl-11 differed from *C. tughillensis* in its dorsiventral vegetative cells. In our multigene phylogeny, *Chloromonas* sp. GsCl-11 was sister to a robust monophyletic group composed of four snow algae strains of *Chloromonas* (including UTEX SNO 91, the authentic *C. tughillensis* strain) within clade A2. Comparing conserved region of ITS2 secondary structures among these five strains, one or more compensatory base changes were found between *Chloromonas* sp. GsCl-11 and other four strains. Thus,

Chloromonas sp. GsCl-11 represents an undescribed species.

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USING DIFFERENT PALEO-SCENARIOS AND A MOLECULAR CLOCK TO EXPLAIN THE ANTITROPICAL DISTRIBUTION OF *SARGASSUM* SUBGENUS *BACTROPHYCUS*

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The genus *Sargassum* is currently subdivided into three subgenera: *Arthrophyucus*, *Bactrophyucus* and *Sargassum*. *Sargassum* subgenus *Sargassum* is abundantly represented in tropical to warm temperate regions of all oceans, while subgenera *Arthrophyucus* and *Bactrophyucus* are recognised to be restricted to temperate and subtropical regions of the southern and northern hemispheres, respectively. Recent taxonomical revision, based on morphological and molecular data, has resulted in the transfer of subgenus *Arthrophyucus* species to subgenus *Bactrophyucus*. Molecular data demonstrated that southern species of *S.* subgenus *Bactrophyucus* sect. *Arthrophyucus* originated in South East Asia and crossed the tropical region only once, resulting in an intriguing antitropical distribution, with a disjunction in the southern hemisphere. We present different scenarios to explain the observed distribution using a multidisciplinary approach, including the calibration of a molecular clock as well as niche and hydrodynamic modelling based on present and paleo-environment conditions.

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RETURN OF THE MAC: DIFFERENTIAL SURVIVAL AMONG EARLY LIFE HISTORY STAGES AND YOUNG RECRUITS OF *MACROCYSTIS PYRIFERA*

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Many field-based studies of macroalgae focus on large conspicuous sporophytes, thereby ignoring the possible effects of ecological processes on the microscopic (and difficult to identify) early life history stages. This is particularly true for the giant kelp, *Macrocystis pyrifera*, a major ecosystem engineer and the dominant canopy-forming alga along the coast of southern California. *M. pyrifera* exhibits a biphasic life history typical of other kelps. However, the majority of field studies on *M. pyrifera* are limited to a single life history stage or are otherwise conducted over short time scales. This study tracked the survival of three different size classes of young *M. pyrifera* (embryonic, 5-10-cm-tall, and 1-m-tall sporophytes) when outplanted to the Point Loma kelp forest, San Diego, CA, at three densities. We also compared the survival of these individuals to those of naturally occurring recruits. With this study, we ultimately examine age-specific variation in density-dependent mortality in the young life-history stages of *M. pyrifera*.

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DIVERSITY AND SPECIES OF *SPIROGYRA* (ZYGNEMATOPHYCEAE, CHAROPHYTA) IN CALIFORNIA IN A MOLECULAR PHYLOGENETIC CONTEXT

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Diversity of the filamentous green algae in the genus *Spirogyra* (Zygnematophyceae) was investigated from more than 1,200 stream samples from California. We identified twelve species of *Spirogyra* not previously known for California, including two species new to science. Environmental preferences of the Californian species are discussed in the light of their restricted distribution to stream habitats with contrasting nutrient levels. We also investigated the systematic relationships of *Spirogyra* species from several continents using two plastid-encoded genes *atpB* and *rbcl*. The molecular phylogeny distributed the Californian species across all the major clades of *Spirogyra* identified in earlier studies, and *Sirogonium* is nested within *Spirogyra*. The phylogeny of *Spirogyra* and its taxonomic implications are discussed, such as the benefits of combining structural

and molecular data for more accurate and consistent species identification. Considerable infraspecific genetic variation of globally distributed *Spirogyra* species was observed across continental scales. This finding suggests that structurally similar species from distant regions may be genetically dissimilar and that *Spirogyra* may contain a large number of cryptic species. Correlating the morphological and genetic variation within the genus will be a major challenge for future researchers.

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SHIFTING STRENGTHS OF SPECIES INTERACTIONS IN A GUILD OF CRUSTOSE CORALLINE ALGAE (RHODOPHYTA, CORALLINALES) RESPOND TO OCEAN ACIDIFICATION

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The crustose coralline algae (Rhodophyta, Corallinales) form an important guild of calcifying primary producers in the temperate Northeast Pacific. In light of documented recent changes in regional ocean carbon chemistry, competition experiments were repeated identical to those performed 1981-1984 among this guild. These experiments quantified interaction strengths of overgrowth competition between crusts (*Lithophyllum impressum*, *Lithothamnion phymatodeum*, *Pseudolithophyllum muricatum*, and *Pseudolithophyllum whidbeyense*) and mediation of competitive interactions by the presence of grazers. In response to pH decline over 30 years, large changes in competitive interactions have led to the reversal of competitive hierarchies among crustose coralline algae. The historical dominant, *P. muricatum*, now wins <25% of its competitive interactions as compared to 100% previously at the same site ($p < 0.001$) and has also become less locally abundant. Further, morphological change in *P. muricatum* is related to its reduced competitive ability. Thallus edge thickness confers a competitive advantage within this guild. Comparison between historical (N=5) and modern (N=6) reveals that *P. muricatum* thallus thickness has decreased by 50% over this interval ($p = 0.031$), directly linking morphological and ecological response to ocean acidification.

ILLUMINATING THE WOUND-INDUCED MACROALGAL OXIDATIVE BURST

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Reactive oxygen species (ROS) are produced upon wounding and pathogen recognition by organisms as distantly related as humans and algae in a defense known as the oxidative burst, and these ROS are typically involved in both direct defense and defense signaling. In vertebrates, the oxidative burst is produced enzymatically by an NADPH-oxidase that interacts with several regulatory proteins. It was recently suggested that the major source of wound-induced ROS in the vascular plant *Arabidopsis thaliana* was disrupted photosynthetic electron transport (PET). However, the source of wound-induced ROS is not well understood in algae, despite the important physiological and ecological consequences. We investigated the effect of light on wound-induced production of ROS by looking at macroalgal oxygen consumption and production of ROS via electron paramagnetic resonance. We discuss the physiological role of light on the macroalgal production of ROS upon wounding as well as the ecological consequences for macroalgal/grazer interactions.

VARIATION OF PHYTOPLANKTON COMMUNITY CHARACTERISTICS OVER CENTIMETER TO KILOMETER SCALES DETERMINED WITH IN SITU OPTICS AND SCANNING FLOW CYTOMETRY

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Phytoplankton abundance, physiology and species composition often vary considerably over centimeter to kilometer scales in the ocean. These patchy distributions of community characteristics are difficult

to study due to the number of observations required to resolve their spatial and temporal structure. As a consequence, our understanding of the fundamental ecological and evolutionary mechanisms that drive phytoplankton growth, mortality, adaptation and speciation has been limited. To address this problem we determined the influence of species composition and intracellular pigment content on the magnitude and spectral shape of absorption and scattering coefficients both in the lab and in the field using scanning flow cytometry, microscopy, and image analysis. We then used in situ optical data to visualize these community characteristics over centimeter to kilometer spatial scales and hourly temporal scales. Results show large variations that demonstrate the importance of small scale processes to the ecology of natural phytoplankton communities. Furthermore, the optical properties of communities appear to be indicative of distinct ecological strategies for light harvesting and may directly relate to the adaptive strategies used by cells within a particular niche.

PHYLOGENY AND TOXICOLOGY OF *LYNGBYA WOLLEI* (CYANOBACTERIA/OSCILLATORIALES) FROM NORTH-EASTERN AUSTRALIA

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Lyngbya wollei (Farlow ex Gomont) Speziale and Dyck is a freshwater benthic filamentous cyanobacterium. Three populations from north-eastern Australia have been putatively identified and found to produce the potent cyanotoxin cylindrospermopsin (CYN) and its analogue deoxy-cylindrospermopsin (deoxy-CYN). *L. wollei* is also a known producer of the potent neurotoxic saxitoxins in the southern United States. We investigated the phylogeny and toxicology of CYN and deoxy-CYN producing *L. wollei* strains isolated from two Australian sites; a small coastal stream, and a large reservoir, using a combination of molecular and morphological techniques. Morphologically the strains from both sites corresponded to the type description, however the frequency of false-branching was low, and variable over time. Samples from both sites were positive for

the *cyrF* and *cyrJ* genes associated with CYN biosynthesis. Phylogenetic analysis of these genes from Australian *L. wollei* sequences and comparable sequences from other CYN-producing cyanobacteria revealed that the genes in *L. wollei* were more closely related to homologous genes in *Oscillatoria* sp. PCC 6506 than to homologues in Nostoclean CYN-producers. This data indicates a common evolutionary origin of CYN biosynthesis in *L. wollei* and *Oscillatoria* for the two genes examined in this study. In both the 16S rRNA and *nifH* phylogenies, the Australian *L. wollei* strains formed well supported clades with other *L. wollei* (= *Plectonema wollei*) strains from the United States. Pairwise sequence similarities within the 16S rRNA clade containing all nine *L. wollei* strains were high, ranging from 97–100%. This group was distantly related (< 92% nucleotide similarity) to other taxa within the group previously considered under the genus *Lyngbya* sensu lato (C. Agardh ex Gomont). Collectively, these results suggest that this toxigenic group is evolutionarily distinct and sufficiently distant as to be considered a separate genus.

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CRYPTIC DIVERSITY AND WHAT IT MEANS FOR *PEDIASTRUM* (HYDRODICTYACEAE, CHLOROPHYCEAE)

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Cryptic diversity within the green algae has resulted in the morphospecies concept being misleading, and impacts the accuracy of utilizing green algae as bioindicators, in biotechnological applications, and for assessing biodiversity. One family for which morphospecies are utilized as bioindicators is the Hydrodictyaceae (Sphaeropleales). A molecular phylogenetic study of the family, based on the nuclear 28S rDNA and chloroplast *rbcL* gene, has revealed cases of hidden genetic diversity of similar morphologies, and close affiliations of distinct morphologies. The genus *Pediastrum* consists of three species, *P. duplex*, *P. angulosum*, and *P. birolinense*. Two separate groups of morphologically similar *P. duplex* isolates are recovered in the molecular phylogeny, one of which contains the morphologically

dissimilar species, *P. angulosum*. The morphologically distinct genus, *Hydrodictyon*, is nested between the two *P. duplex* groups. Newly collected targeted sequence data from the chloroplast genes *psaA* and *psaB* support the findings of cryptic diversity within the family, indicating the need for genomic data to assist with clarifying the species relationships and how best to define the genus *Pediastrum*.

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WILL THE REAL PICOBILIPHYTE PLEASE STAND UP?

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Using vital mitochondrial staining and cell sorting by flow cytometry, a culture of a “picobiliphyte” has finally been established. This clonal culture, which has a ‘picobiliphyte’ 18S rDNA signature, is neither pico or pigmented. Its morphological and ultra-structural characters clearly show that it is slightly elongate and 2-5 µm in length with two unequal flagella not covered by hairs or scales. It exhibits unique cell movements (jump, drag, and skedaddle mode of locomotion) but often remains suspended in the water column for extended periods. Light and electron microscopic studies reveal that the cells are naked and that in this isolate, a plastid is lacking. A unique feeding apparatus suggests a rather rare mode of feeding on DOM is possible. The cells in this culture, thus, are heterotrophic, although their food source could not be determined, and food vacuoles containing bacteria were never observed. The cells harbor several other unique compartments that do not match those in any other known eukaryotes. This uniqueness is corroborated by phylogenetic analyses of the complete nuclear ribosomal operon, although the clade containing “picobiliphyte” sequences has some affinity with the Hacrobia. With their cell morphology now firmly established, we can now formally describe an important and abundant member of the eukaryotic pico/nanoplankton. This group/division is placed in a new phylum ‘Picozoa’ with *Picomonas judraskeda* as its type species.

MIDTAL: MICROARRAYS FOR TOXIC ALGAE AND MICROAQUA: MICROARRAYS FOR FRESHWATER PATHOGENS

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Microalgae in marine and brackish waters of Europe regularly cause harmful effects, considered from the human perspective, in that they cause economic damage to fisheries and tourism. Cyanobacteria cause similar problems in freshwaters. These episodes encompass a broad range of phenomena collectively referred to as “harmful algal blooms” (HABs). For adequate management of these phenomena, monitoring of microalgae is required. However, effective monitoring is time consuming because morphology as determined by light microscopy may be insufficient to give definitive species and toxin attribution. In the EU FP7 project MIDTAL (microarrays for the detection of toxic algae) we achieved rapid species identification using rRNA genes as the target. These regions can be targeted for probe design to recognize species or even strains. We also included antibody reactions to specific toxins produced by these microalgae because even when cell numbers are low, toxins can be present and can accumulate in the shellfish. Microarrays are the state of the art technology in molecular biology for the processing of bulk samples for detection of target RNA/DNA sequence. After 45 months we have completed RNA-cell number–signal intensity calibration curves for 18 HAB species, and the analysis of monthly field samples from five locations from two years of field data. Results from all partners will be presented. A new project microaqua is introduced. A commercial kit for MIDTAL is now available for testing.

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A MOLECULAR INVESTIGATION OF *ULVA* (ULVOPHYCEAE, CHLOROPHYTA) IN THE GULF OF MEXICO BASED ON THE CHLOROPLAST MARKERS *RBCL* AND *TUFA*

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The green algal genus *Ulva* is found all over the world in marine, brackish, and sometimes freshwater environments. Certain *Ulva* species cause ecologically and economically destructive green tides in eutrophic conditions. Morphology-based identifications of species within the green algal genus *Ulva* are infamously difficult, and incorrect circumscriptions of these species have created a taxonomic conundrum since the genus was first recognized by Linnaeus in 1753. Fortunately, molecular studies based on the nuclear-encoded ITS marker and chloroplast-encoded *rbcL* and *tufA* markers have provided a foundation for a molecular species concept. Previous molecular studies have been completed in many regions around the world; however, molecular data are not available from the Gulf of Mexico. A molecular approach based on the chloroplast-encoded *rbcL* and *tufA* molecular markers is providing a better understanding of the biodiversity of *Ulva* in the Gulf of Mexico. Our molecular analyses confirm four out of the twelve species previously reported based on morphology. Additionally, these results are revealing an unexpected cryptic diversity and the presence of *Ulva ohnoi*, a green tide species and new report for this region.

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DIVERSITY OF PLANKTONIC ALGAE IN A COASTAL WETLAND “LA ENCANTADA”, PERU

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Peru is a country with high levels of recorded biodiversity. The biodiversity of microorganisms, however, is very poorly known. The objective of this study was to determine the planktonic algal composition from La Encantada, specially focused on cyanobacteria diversity. The study was conducted in March, August and October 2012. The samples were collected with a plankton net (20 µm) and then fixed with 4% formaldehyde. Physicochemical variables were measured *in situ*. The algal samples were treated with India ink and Lugol’s iodine; diatoms were oxidized and mounted in Zrax. 26 taxa were identified, of which 5 are new records for the Peruvian algal flora: *Planktothrix* sp., *Prymnesium* sp., *Kirchneriella*

contorta, *Coelomon pusillum*, and *Coelomon tropicale*. In addition, we recognized 6 cyanobacterial taxa that don't match any previously described species. We registered blooms of *Peridinium* sp., *Microcystis* sp., and a filamentous cyanobacterium that was not identified because it didn't match with any described in the literature. The possibly toxicity of some cyanobacteria is latent, especially when taking into account the changes in temperature and precipitation in recent years due to global warming.

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**A NEW PATHOGENIC FUNGUS
ALERNARIA SP. CAUSED RED ROT
DISEASE OF PYROPIA/PORPHYRA
YEZOENSIS CULTURED IN NORTHERN
CHINA**

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Pyropia/Porphyra yezoensis (nori) is an economically important red seaweed, which was cultivated in large scale along the coast of Northern China. During the early winter of 2011, a large-scale and serious red-rot disease broke out throughout the whole nori cultivation area of China. It is estimated that 40-50% of annual production was lost. This disease initially appeared with needle size red spots in the thallus. Holes were then formed by the growing spots, which finally led to the loss of the rotted fronds. In order to investigate the causative agent of the red-rot disease, we monitored infected *Pyropia* plantations from several distant loci in Shandong Province and Jiangsu Province and isolated the pathogen. We found that the "red-rot" diseases in all sites were caused by the same endoparasitic fungus, assignable to the genus *Alternaria* based on morphological and molecular-phylogenetic analysis. Pathogenicity assay indicated this fungus was able to cause the typical red-spot symptoms seen to occur naturally. This is the first report of the species of *Alternaria* sp. that is able to cause the disease in seaweed.

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**ADAPTATION TO POLAR SEA ICE
FACILITATED BY ALLELIC**

**DIVERGENCE IN A PSYCHROPHILIC
DIATOM**

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Diatoms contribute 25% of global carbon fixation, but their significance is especially pronounced in cold, nutrient-rich polar oceans. Many diatom species were even able to adapt to sea ice that covers about 7% of the Earth's surface. How diatoms have evolved tolerance to this extreme, ephemeral sea ice habitat is largely unknown. Here we present insights into the genome evolution of the psychrophilic diatom *Fragilariopsis cylindrus* that is a keystone diatom species of Southern Ocean sea ice. Evidence from comparative genome, transcriptome and metatranscriptome analyses revealed a unique diversification of ~33% of all genes in the genome of *F. cylindrus*, resulting in a significant enrichment in metabolism, intracellular protein transport and lipid metabolism. Alleles of the same genes respond differently to environmental stimuli, and their homologs account for 73% of all *F. cylindrus*-specific transcripts in natural sea-ice communities, including the most highly abundant transcripts. Obligate clonal reproduction avoids the 'segregation load' caused by recombination and the formation of homozygous genotypes. These data give strong evidence that the loss of sex allowed for the functional divergence of alleles, which enabled *F. cylindrus* to adapt to the extreme and variable conditions in polar sea ice of the Southern Ocean.

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**MORPHOLOGICAL DIVERSITY OF
CYANOBACTERIA AND MICROALGAE IN
THE COASTAL LOMAS FORMATIONS IN
THE PERUVIAN DESERT**

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The Peruvian tropical desert is located at the central western coast of South America. Wet sea-fog drifts landward settling on coastal hills of the lomas formations such as Iguanil and Pachacamac in Lima

Department. Standard cyanobacterial and microalgal collections from the aeroterrestrial habitats with dry or moist soil crusts were carried out in 1997 and 2011. Cyanobacterial and algal isolation and cultivation of the species were obtained in BBM and DSN media. Registered in the floristic survey are the eukaryotic terrestrial algae *Cylindrocystis brebissonii* (Zygnematophyceae, Streptophyta), *Klebsormidium* cf. *bilatum* (Charophyceae, Streptophyta), *Closteridium* sp. (Chlorophyceae, Chlorophyta) and the cyanobacteria *Plectonema tenue*, *Nostoc commune*, *Microcoleus vaginatus*, *Scytonema myochrous* and *Pleurocapsa entophysaloides*. *N. commune* was dominant and its desiccation tolerance (summer and early fall) evidenced their long-term survival. Macroscopic growth habit, vegetative propagation and sexual reproduction phases of species life cycle are related to the annual fluctuating garua (seasonal fine precipitation) and desiccation periods. Their adaptive strategies (mucilage, cell wall thickness, spores) provide viable life strategy for ecological success.

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**THE IN SITU GROWTH RATE
EXPERIMENT OF DINOFLAGELLATES
BY SIZE FRACTIONATED METHOD
ALONG KARACHI COAST**

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The *in situ* growth rate experiment of dinoflagellates was studied by the size-fractionation method along the Karachi coast off Pakistan. The field samples were collected during winter (February 2006) and summer (May 2007). Growth rates (μ_{max}) were variable and ranged between -2.87 to 2.3 day^{-1} (20 species) in winter and 1.20 to 1.95 day^{-1} (13 species) during summer. Growth rates (μ_{max}) of dominant species were *Prorocentrum gracile*, *P. minimum*, *P. arcuatum* ($\mu_{max} = 1.0 - 1.10 \text{ day}^{-1}$), *Protoperidinium steinii* (0.92 day^{-1}), *Gonayualax spinifera* (0.69 day^{-1}), *Dinophysis acuminata* (2.3 day^{-1}), *D. caudata* (0.92 day^{-1}), *Ceratium lineatum*, *P. micans* (1.95 day^{-1}), *Gyrodinium* sp. (1.88 day^{-1}), *C. furca* (1.70 day^{-1}), *Alexandrium ostenfeldii* (1.34 day^{-1}). Our results suggested that maximum growth is obtained at high

temperature and low Chl *a* concentration during summer.

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**EFFECTS OF IRON ENRICHMENT ON
TROPICAL MARINE ALGAE,
TETRASELMIS TETRAHELE AND
BOERGESENIA FORBESII**

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Iron fertilization has been proposed as a method for reducing ocean acidification due to climate change. We investigated the effect of iron enrichment on a tropical marine microalgae *Tetraselmis tetrahele* and a seaweed *Boergesenia forbesii*. Effects on growth (chlorophyll *a*, carotenoid), biochemical composition (carbohydrate, protein and lipid), DNA damage (Random Amplified Polymorphic DNA (RAPD)) and Superoxide dismutase (SOD) enzyme activity, after short term (four days) and long term (ten days) exposure were studied. Results showed that the carbohydrate, protein and lipid content decreased with increasing concentration of iron. The SOD activity in the exposed algae increased with increasing iron concentration until the threshold, beyond which the cells lost their resistance and died. Changes occurred in the RAPD profiles (variation in band intensity as well as gain or loss of bands) in all treated samples compared with control. Findings from this study will contribute towards the development of bioassays for the detection and monitoring of Fe contamination based on DNA damage detection, growth, biochemical composition and stress enzyme response in tropical algae.

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**CONTRIBUTION OF CALCIUM TO THE
CELL WALL STRUCTURE IN THE
BROWN ALGA, ECTOCARPUS
SILICULOSUS**

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The brown algal cell wall is composed mainly of cellulose, alginate and fucoidan, but also contains proteins and phenolic compounds. It has been known that calcium ions contribute to the nature of the cell wall compartment of brown algae, especially in alginate mechanics. To clarify the role of calcium ions, observations by electron microscopy and proteomic analysis were performed on the filamentous brown alga *Ectocarpus siliculosus*, when grown in media containing different concentrations of calcium ions. The cell wall of *Ectocarpus siliculosus* consists of three distinct layers. Ultrastructural observations showed that the layered structure disappeared, and mesh of fibers in the cell wall became looser in low calcium medium. Disorder of the cell wall integrity was also promoted by addition of sorbitol. In proportion to reduction of calcium in medium, rupture of cell wall and production of protoplasts were induced. Soluble proteins released into the medium under these conditions were analyzed using LC-MS/MS, and proteins involving alginate synthesis, signal transduction and redox regulation were detected. It was considered that proteins, which were extracted with low-calcium concentration, were loosely bound to the cell wall architecture.

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INVESTIGATION OF THE *GRACILARIA GRACILIS* PROTEOME RESPONSE TO NITROGEN LIMITATION

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Gracilaria gracilis, a commercially important red macroalgal species, is used extensively in the production of agar. Nitrogen limitation has been identified as the major factor limiting growth of *G. gracilis* populations in South Africa. While the physiological mechanisms implemented by *G. gracilis* are well understood little is known about the molecular mechanisms. In this study, a differential proteomics approach employing two-dimensional gel electrophoresis and liquid chromatography tandem mass spectrometry was used to investigate *G. gracilis* proteome changes in response to nitrogen limitation. Putative identities of 16 proteins that were found to be differentially regulated ($P < 0.05$) were obtained. The

identified proteins functioned in a range of biological processes including glycolysis, photosynthesis, ATP synthesis, protein-refolding and biosynthesis, and cytoskeleton remodelling. Fructose 1, 6 biphosphate aldolase, observed to be differentially regulated in response to nitrogen limitation, was further characterised. The decreased abundance of fructose 1, 6 biphosphate aldolase was validated by enzymatic and western blot analysis. This study provides the first characterisation of *G. gracilis* proteome changes in response to nitrogen limitation and identifies key proteins involved in this response.

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A SPECIFIC ANTIFOULING STRATEGY OF *SARGASSUM SILIQUASTRUM* (PHAEOPHYCEAE) AGAINST EPIPHYTISM OF *NEOSIPHONIA HARVEYI* (RHODOPHYTA)

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Our previous study revealed that the epiphytic red alga *Neosiphonia harveyi* is remarkably less abundant on *Sargassum siliquastrum* than *Myagropsis myagroides*, *S. hemiphyllum*, *S. patens*, and *S. piluliferum*, all of which are main components of large algal beds in Wakasa Bay, Japan. Although *S. siliquastrum* may have some inhibitory factor against this epiphyte, such an antifouling mechanism is unknown. First, we qualitatively analyzed phlorotannins of the above five sargassacean algae, which have been reported to show an inhibitory effect against several epiphytes, using NMR. As a result, phlorotannin components of *S. siliquastrum* were not remarkably different from those of the other four species. Second, carpospore settlement and germination of *N. harveyi* were assessed using the PES media dissolving various concentrations of phlorotannins. A sufficient inhibitory effect was demonstrated only in the highest concentrations (1 g/L) and was not significantly different among the five sargassacean species. Third, we performed SEM observations of surface structures because cuticle peeling has been considered effective for preventing epiphytes. The degree of cuticle peeling, however, varied even within the same species

and did not seem different among the five species. Finally, sargassacean blades were incubated with spores of *N. harveyi* to observe the antifouling process. Many of the spores were attached and germinated on the blades of every species, but the blade tissues of *S. siliquastrum* where sporelings attached were swollen and then removed together with sporelings. This phenomenon was not seen on the blades fixed in ethanol, suggesting vital reaction of *S. siliquastrum* against this epiphyte. It is, therefore, considered that this antifouling mechanism is more efficient to prevent *N. harveyi* than the conventional chemical or physical defenses.

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PHYLOGENETIC SIGNAL, NOISE AND THE PHYLOGENY OF CYMBELLALES (BACILLARIOPHYCEAE)

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We studied phylogenetic signal and noise in a newly assembled dataset for the phylogeny of the Cymbellales (Bacillariophyceae). Despite a substantial increase in the amount of data and expanded taxon sampling, we found poor support crucial nodes in the Cymbellales tree. To assess whether this outcome is due to noise from fast-evolving sites we characterized the evolutionary rates with and without a phylogenetic tree. We find that the sequenced genes exhibit bimodal distributions of tree-independent evolutionary rates concentrated in the low- or high-rate ranges.

Phylogenetic informativeness of the dataset peaks before the ages of poorly supported deep divergences thus has a high probability of introducing noise. Characters of different quality, perhaps helpful for resolution of early nodes, are lacking. Rate-based partitioning strategies simplify and improve the phylogenetic model but provide only marginal improvement of clade support. Removal of noisy characters, albeit significantly reducing the amount of noise, has a costly outcome of removing large quantity of signal. Ingroup taxon sampling targeted at breaking up long stems with uncertain/unsupported

phylogenetic placement will likely yield more robust estimates of the Cymbellales phylogeny.

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HETEROKONTOPHYTA (PHAEOPHYCEAE) AND CHLOROPHYTA FROM BIMBARRAS ISLAND, TODOS OS SANTOS BAY, BAHIA, BRAZIL

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The present work includes an inventory of the Heterokontophyta (Phaeophyceae) and Chlorophyta from Bimbarras Island in the municipality of São Francisco do Conde, in the northern region of Todos os Santos Bay, Bahia, Brazil. Submerged sandstone reefs, non-consolidated sandbanks and extensive areas of mangrove swamp are encountered on the island. The study material was collected every two months during the period between September/2008 and March/2012. All of the material was examined using a light microscope and the samples were incorporated into the HUEFS collection. A total of 61 taxa were identified. The division Chlorophyta demonstrated the greatest representivity, with 42 taxa; the orders Bryopsidales and Cladophorales and the families Caulerpacae, Cladophoraceae, Codiaceae and Ulvaceae demonstrated the greatest relative floristic richnesses. Among the representatives of the division Heterokontophyta, the orders Dictyotales and Ectocarpales were the most numerous; the family Dictyotaceae had the greatest number of taxa (eight), followed by the families Scytosiphonaceae (with three taxa) and Acinetosporaceae (with two taxa). As a result of this study, *Neomeris mucosa* is recognized as a new addition to the marine flora of the American Southern Atlantic, and earlier records of *Dictyota implexa* along the southern coast of Brazil were confirmed.

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HYPNEA FROM BRAZIL: DIVERSITY, MOLECULAR PHYLOGENY AND TAXONOMY

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The genus *Hypnea* Lamouroux (1813) (Cystocloniaceae, Rhodophyta) has great ecological and economic importance as source of carrageenan, but the species are morphologically simple, with large phenotypic plasticity, which complicates their identification on solely morphological data. Due to these taxonomic problems, in this work we sequenced two short molecular markers, the mitochondrial 5'-cox1 and plastid UPA, plus the plastid marker rbcL for phylogenetic analysis, associated with morphological studies to unravel the diversity of the genus to the coast of Brazil. We obtained 200 *Hypnea* samples collected in the intertidal and subtidal zone. Based on molecular and morphological data we found ten taxa of *Hypnea*: *H. cenomyce*, *H. cervicornis*, *H. cornuta*, *H. flexicaulis*, *H. musciformis*, *H. spinella*, *H. stellulifera*, *H. volubilis*, *H. sp1* and *H. sp2*. We conclude that *H. nigrescens* in Brazil is a morphological variation of *H. musciformis*. This is the first mention of the species *H. stellulifera* for the South Atlantic. The results indicated that there is a significant number of cryptic species in the genus, which can be identified using the "DNA barcoding" technique.

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SYSTEMATICS OF SECTION *TURFOSA* OF THE GENUS *BATRACHOSPERMUM* (BATRACHOSPERMALES, RHODOPHYTA)

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The section *Turfosa* has been a well-supported clade in molecular studies of the Batrachospermales, but only a few sequences have been utilized. The goal of the present research was to document the number of species and their relationships using the *rbcL* gene and COI barcoding region. Thirty-seven COI and 22 *rbcL*

DNA sequences were generated from North American, European, and South American (primarily Brazil) specimens. Eight haplotypes/species were found. There were three haplotypes identified from North America as follows: one haplotype from a few geographically restricted locations, one widespread including northern South America and a third collected from a single location, but this haplotype was common in Europe. Of the three haplotypes in Europe, one was widespread and the other two more restricted. Three haplotypes occurred in Brazil. Analysis of morphology allowed three species epithets *B. keratophytum* Bory, *B. turfosum* Bory and *B. orthostichum* Skuja to be placed on the clades; all others represent new species. In addition to the description of this previously unrecognized diversity, the section *Turfosa* will be raised to genus level.

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A COMPARISON OF DIATOM COMMUNITIES IN LIVING AND DEAD SPHAGNUM MOSSES

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An ongoing biodiversity research on the algal flora of numerous bogs of the "Vepssky Forest" Natural Park, Leningrad region, Russia, revealed two peat bogs, separated by 36 km, with several patches (~2 m) of black disintegrating *Sphagnum*: a wet *Sphagnum* bog, found in 2006, and a bog adjoining a small lake. These patches differed from surrounding bogs, where diverse communities typical for peat bogs were present, dominated by *Frustulia krammeri* Lange-Bertalot and Metzeltin, *Eunotia exigua* (Brebisson in Kutzing) Rabenhorst, and *E. arcus* Ehrenberg. In the black patches there were only a few, abundant species. In both bogs the dominant species was *Kobayasiella parasubtilissima* (Kobayasi & Nagumo) Lange-Bertalot, which is an indicator of acidic and oligotrophic conditions. It was accompanied by *Eunotia paludosa* Grunow and *E. sp.1* (first bog) and *E. microcephala* Krasske and *E. sp.2* (second bog). *K. parasubtilissima* wasn't confined to the patches, but was rare elsewhere. Buczko et al. (2009) described the distribution of *Kobayasiella* taxa in European bogs as sporadic and unpredictable. It seems that *Kobayasiella*

is tied to something else in bogs, rather than oligotrophic conditions.

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BIOMASS AND GROWTH DYNAMICS OF SACCHARINA LATISSIMA IN A TEMPERATE EMBAYMENT

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Seasonal growth patterns influence the turnover of organic matter and the structure of the kelp habitat and are, therefore, an important aspect of the role of kelp in coastal ecosystems. Being tightly coupled to abiotic growth conditions, seasonality is likely to change with latitude. We described seasonal growth, loss and nutrient dynamics of *S. latissima* in Denmark (56°N) and compared the findings with previous studies (41-74°N) along the geographical distribution range of the species. Length growth peaked in March-May and biomass growth in May-July while major biomass losses took place July-September. This seasonality caused a biomass turnover of the population of 1.2 year⁻¹. Seasonal nitrogen content showed maximum and minimum values in March and September respectively. The opposite trend was found for carbon content. Maximum length growth rates (7.5 ± 0.4 mm d⁻¹, March-May) fell in the central part of the global dataset which generally exhibited higher and earlier timing of maximal growth rates with decreasing latitude. However annual length growth (132 cm) fell in the lower range of other studies indicating that local factors also play important regulating roles.

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PHYTOPLANKTON ASSOCIATIONS IN THE VICINITY OF DESOTO CANYON, NORTHEASTERN GULF OF MEXICO: SOME PRELIMINARY COMMENTS ON DISTRIBUTIONAL PATTERNS

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We are conducting a multi-year study of phytoplankton in the vicinity of DeSoto Canyon, northeastern Gulf of Mexico. So far, 16 cruises have been conducted, with additional cruises planned at quarterly intervals through 2014. Samples are collected from 27 stations along three transects extending ~80 km from the coast. At each station 1-liter samples are collected at regular intervals to depths of up to 200 m. These are filtered onto 0.45 µm filters and examined with SEM to quantify abundant species. Net-plankton (25 micron mesh) samples are collected at odd-numbered stations for the analysis of larger forms. So far 35 prymnesiophyte genera, 55 diatom genera, and 6 dinoflagellate genera have been documented. Important groups include the diatom families Bacillariaceae, Chaetocerotaceae, Leptocylindraceae, Rhizosolenaceae, and Thalassiosiraceae, and the prymnesiophyte *Emiliania huxleyi*; in addition, the diatom genus *Nanoneis* is common at off-shore stations. While diatoms generally dominate the association, the dinoflagellate genera *Ceratium*, *Dinophysis*, and *Prorocentrum* were the most abundant forms in net samples from March 2011. Numbers of individual cells in surface waters decrease with increased distance from the shore. Off-shore stations can develop a deep chlorophyll maximum (DCM) at depths in excess of 40 meters. Within a profile, the composition of the DCM is distinct from that of the surface, usually with a greater number of taxa, and often with a higher concentration of cells.

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IODINE CONTRIBUTES TO OSMOTIC ACCLIMATISATION IN THE KELP LAMINARIA DIGITATA (PHAEOPHYCEAE)

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Laminaria digitata retains inorganic iodide at levels 30,000 times those present in seawater but the physiological function of iodine remains largely underexplored. It is known that osmoregulation in *L. digitata* is achieved by adjustment of the intracellular mannitol concentration but high iodide levels could also contribute to osmotic homeostasis. Here, impacts of salinity on iodine uptake by, and release from, *L. digitata* were assessed. The accumulation of mannitol was also determined and photo-physiological

responses were measured by chlorophyll fluorometry. Iodine was efficiently taken up at both normal and an increased salinity of 50 SA, but at 50 SA the presence of iodine appeared to inhibit mannitol accumulation. High tissue iodine concentrations were accompanied by elevated NPQ_{max} and high Fv/Fm. By contrast, low tissue iodine levels at 50 SA coincided with reduced NPQ_{max} and photoinhibition, although mannitol was accumulated. Salinity also controlled the iodide release into seawater and lowest release rates were observed at highest salinities. The results suggest that under high salinity stress the incorporation of inorganic iodine is probably more efficient and energetically less expensive than synthesising mannitol.

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FINE STRUCTURE OBSERVATION DURING SILICEOUS SHELL FORMATION OF A TESTATE AMOEBA *PAULINELLA CHROMATOPHORA* (EUGLYPHID)

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Paulinella chromatophora is a testate amoeba (euglyphid) with a pot-shaped siliceous shell. It is known to form silica scales within the cell and build the shell for a daughter cell before cell division. Using a time-lapse video observation, we have reported that: 1) all scales were secreted first and rearranged outside of the cell; 2) the scales were then piled up one by one using a thick pseudopodium to form the shell; 3) then one of daughter cells moved into the new shell. However, the detailed process of shell construction is still a mystery. In this study, we describe the fine structural processes of scale formation and shell construction in *P. chromatophora*. We identified a silica-deposition vesicle (SDV) in the cell and observed the detailed developmental process of scales. We also found that, during the shell construction, the expanded front edge of the thick pseudopodium branched and held all scales that were waiting to be assembled. Only several mitochondria, vesicles containing dense material, and tubular membranes that seemed to be highly branched were observed in the thick pseudopodium.

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A NEW ‘MISSING LINK’ GENUS OF THE COLONIAL VOLVOCINE GREENS GROWING IN JAPANESE AND AUSTRIAN FRESHWATERS

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The volvocine algae from unicellular *Chlamydomonas* to multicellular *Volvox* represent an “evolutionary time machine” for studying the evolution of female-male sexual dimorphism and the multicellularity because they encompass the entire ranges between these two extremes. On the basis of chloroplast multigene phylogeny, a major evolutionary scenario within the lineage was resolved ranging gradually from primitive unicellular organisms with isogamy to the advanced multicellular spheroid colonies with oogamy such as *Volvox*. This group contains an enigmatic genus *Platydorina* with flattened vegetative colonies developing via unique embryogenesis “intercalation.” However, the evolutionary scenario of origin of *Platydorina* has remained unresolved possibly because of the lack of a robust sister lineage, resulting in ambiguous phylogenetic resolutions, even when using multiple chloroplast. Here we described a new colonial volvocine genus, which may represent a missing link between *Platydorina* and the typical spheroidal volvocine algae. Our chloroplast multigene phylogeny resolved that the new genus is robustly sister to *Platydorina*. This new genus has 16- or 32-celled spheroidal colonies that are similar to those of the volvocine genera *Yamagishiella* and *Eudorina*. However, it can be clearly distinguished from the latter two genera by its two or three contractile vacuoles present only in the anterior portion of each vegetative cell and by possible anisogamous sexual reproduction; these two features are similar to those of its sister genus *Platydorina*. Albeit of rare occurrence, two species of the new genus were found in a Japanese lake, and one of the two possibly grows in an Austrian freshwater.

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**PROACTIVE ASSESSMENT OF
ALGICIDES FOR RAPID RESPONSE TO
POTENTIAL INVASIONS BY AQUARIUM
STRAINS OF THE SEAWEED
CHAETOMORPHA**

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Biological invasions associated with home/public aquaria releases are an issue of global concern. The genus *Chaetomorpha* is currently the most popular macroalgal group for saltwater aquariums, used by over 50% of surveyed hobbyists in the US. As such, the opportunity for its introduction is considerable and poses a serious threat to aquatic systems. We tested five chemical algicide treatments—chlorine bleach, rock salt, copper sulfate, acetic acid and Sonar (active ingredient fluridone)—to determine the best techniques for rapid eradication or control of an invasion by aquarium *Chaetomorpha*. We conducted factorial experiments of 1-cm fragments from 3 retail purchases of *Chaetomorpha* exposed to experimental concentrations of each algicide for varying durations, rinsed the fragments, and then resubmerged them in algicide-free artificial seawater. We monitored survival and growth for 4 weeks following the end of chemical exposure. Only acetic acid was consistently effective at inducing full mortality at tested exposure levels (at least 2% for 4 min). Chlorine bleach results were inconsistent across purchases. Rock salt and copper sulfate did not significantly reduce survival but limited growth.

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**SCREENING OF CAROTENOIDS FROM
POTENT MICROALGAL STRAINS OF
INDO- BIODIVERSITY HOTSPOTS AND
PROCESS OPTIMIZATION FOR
COMMERCIALIZATION**

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Northeast India (22-30°N and 89-97°E), spreading over 2,062,379 sq km, falls under the Indo-Burma Hotspot

of the 34 Hot Spots of the world identified in India. It represents the transition zone between the Indian, Indo-Malayan and Indo-Chinese biogeographical regions and acts as a meeting place of the Himalayan Mountains and Peninsular India. It is characterized by a rich bio-diversity and heavy precipitation, providing a perfect platform for the exploration and exploitation of microalgae. The National Facility for Freshwater Cyanobacterial and Microalgal Repository of IBSD, Imphal, Manipur, India created by Department of Biotechnology, Govt. of India, harbors about 1636 strains of microalgae from the eight states of the north east region of India. All available strains are taxonomically and biochemically characterized and identified up to genus/species level and maintained as unialgal cultures with accession number. In the present study, 300 unialgal isolates were screened for quantification of available carotenoid. Out of these *Leptolyngbya* sp. BTA-287, an isolate from West Agartala, Tripura, India, was found to contain highest level of carotenoids (25.38±0.007 µg/mL) during log phase while *Phormidium* sp. BTA- 412 from Imphal East, Manipur, India, showed the highest level of carotenoids content (24.58±3.27 µg/ml) during declining phase of growth. The process optimization for enhancement of carotenoid content through biotechnological intervention, or the application of nutritional and ecological features yielded exciting results, but needs to be established for a longer period before the technology can be commercialized. These cultures were studied extensively for other bioactive molecules as well as characterized morphologically and identified at the molecular level using 16S rRNA.

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**ENGINEERING CHARACTERIZATION OF
A SHAKEN MICRO-BIOREACTOR
PLATFORM FOR PARALLEL
EVALUATION OF MICROALGAE
CULTIVATION AND SCALE-UP**

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Micro-scale technologies for early prediction of key bioprocess parameters and scale-up have previously been applied to biopharmaceutical production and production of human cells for therapy. However, their application to sustainable, bio-renewable energy

bioprocess development is only now being considered. Here we describe a shaken micro bioreactor platform that provides the required biochemical engineering conditions suitable for optimization of microalgae cultivation conditions and scaled up to 7.5 L bioreactor. Cells are cultured in up to 24 parallel wells, shake flasks and 7.5 L bioreactor with working volumes of 4 mL, 100 mL and 4000 mL respectively. Characterization of a biochemical engineering conditions suitable for scale up were based on the mixing time (Tm) and oxygen mass transfer coefficient (kLa) studies. Parallel batch culture was performed for the three systems at matched kLa corresponding to 300-350 rpm, with 80 % relative humidity as appropriate conditions for evaporation and condensation control in the shaken incubator. However, impact of the engineering conditions (1 vvm air flow rate and 300 rpm shaking speed) on the growth kinetics for *Chlorella sorokiniana* showed similar biomass concentration of about 5 g L⁻¹, pigment production, pH, glucose depletion and total lipid yield with high fatty methyl esters acid required for bioenergy production. Adoption of the new micro-scale shaken bioreactor platform will enable rapid and parallel evaluation of multiple strains of microalgae for their biofuel and high value chemicals production capabilities. This will help reduce bioprocess development costs, time and risks.

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EFFECTS OF OCEAN ACIDIFICATION ON DIFFERENT LIFE-CYCLE STAGES OF THE KELP *LAMINARIA HYPERBOREA* (PHAEOPHYCEAE)

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The objective of this study was to evaluate the influence of preindustrial and expected future atmospheric CO₂ concentrations (280 μatm and 700 μatm pCO₂, respectively) on different life-cycle stages of the kelp *Laminaria hyperborea* from Helgoland (Germany, North Sea). Zoospore germination, gametogenesis, vegetative growth, sorus formation and photosynthetic performance of vegetative and fertile tissue were addressed. The contribution of the external carbonic anhydrase

(exCA) to C-supply for net-photosynthesis and the Chl *a* and phlorotannin content were examined. Female gametogenesis and vegetative growth of sporophytes were significantly enhanced under the expected future pCO₂. rETR (max) and net-photosynthesis of young vegetative sporophytes displayed a trend towards increased performance at higher pCO₂. The trend towards elevated net-photosynthesis vanished after inhibition of the exCA. In vegetative sporophytes, phlorotannin content and Chl *a* content were not significantly affected by pCO₂.

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PEERING INTO THE POND FOR CLUES TO MULTICELLULARITY

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The evolution of multicellular organisms is a major evolutionary state transition that has occurred at least twenty-five independent times in all domains of life. However, the molecular-genetic and ecological basis of multicellular evolution remains enigmatic. Classically, the Volvocacean algae, which include the well-known algae *Chlamydomonas reinhardtii* and *Volvox carteri*, have been used as an important model system for multicellular evolution. The Volvocales are an important model of multicellular evolution for many reasons including: 1) they represent the most recent known example of multicellular evolution; 2) because the genomes of the organisms are very closely related, and 3) because they are a tractable molecular-genetic model system. The first step for multicellular organisms to evolve occurs when is when unicells for a collective, termed colonialism. Because colonialism is the first step where organismal selfishness is lost, it is widely viewed to be the rate limiting step of multicellular evolution. In the Volvocacean algae, *Chlamydomonas reinhardtii* represents the unicellular state, while the closely related alga, *Gonium pectorale*, represents the transition to colonialism. Significantly, colonialism in the Volvocacean algae evolved by the modification of the cell cycle such that mitotic daughter cells remain attached together after mitosis. Significantly, in the draft genome sequence of *Gonium pectorale* it is observed that the retinoblastoma tumor suppressor cell cycle regulatory pathways have undergone significant modification compared to

Chlamydomonas reinhardtii. Finally, it is often assumed the unicellular organisms do not cooperate, however, our recent findings suggest that *Chlamydomonas reinhardtii* cooperates in the face of environmental challenges, suggesting that the path to cooperation in a multicellular organism may be simpler than originally thought.

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ULTRASTRUCTURAL AND PHYLOGENETIC STUDIES OF THE UNARMORED KLEPTOCHLOROPLASTIDIC DINOFLAGELLATES

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‘Kleptochloroplast’ is a temporary chloroplast ‘stolen’ from other photosynthetic algae. The unarmored dinoflagellates *Amphidinium poecilochroum* and *Gymnodinium aeruginosum* are closely related to each other, and both possess cryptomonad-derived kleptochloroplasts. *G. aeruginosum* can synchronize its kleptochloroplast division with that of its own, while no such phenomenon occurs in *A. poecilochroum* – the situation indicates that kleptochloroplastidy in the former is at a more advanced stage in acquiring a ‘true chloroplast’. Because the fate of the ingested cryptomonad remains unclear, we studied the ultrastructural changes of the kleptochloroplast in both species. This revealed that *A. poecilochroum* removed most of cryptomonad organelles very early on, while *G. aeruginosum* retained many of the cryptomonad organelles, including the nucleus. This suggests that the retention of cryptomonad nucleus is an important step in realizing an advanced state of kleptochloroplastidy. In addition, we discovered two novel heterotrophic dinoflagellates that form a robust clade with the unarmored kleptochloroplastidic dinoflagellates. This study suggests that these heterotrophic dinoflagellates are at an evolutionary stage at which they either have lost or have yet to acquire the ability of kleptochloroplastidy.

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MACROALGAE DIVERSITY AND DISTRIBUTION IN CORAL REEFS OF THE

SOUTHERN MEXICAN CARIBBEAN

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This study investigates the distribution and diversity of benthic macroalgae in coral reefs of two locations (Mahahual and Xahuayxol) in the Southern Mexican Caribbean. Data were collected per location – within 5 quadrants along two 50 m transects – at three areas: reef lagoon, reef terrace and reef slope. A presence-absence matrix was built per location and area, and the diversity was compared through the Jaccard similarity analysis. A total of 139 macroalgae species was found (78 Rhodophyta, 13 Phaeophyta, 36 Chlorophyta, 3 Cyanobacteria). At least 73 species were found in Mahahual (with higher species richness in the reef slope north) and 125 in Xahuayxol (with higher species richness in the reef terrace south). In general, Xahuayxol showed the highest diversity, but no Jaccard similarity was found among locations. There was no difference in species richness (t-student, $p > 0.05$). Differences may only be explained at species composition level, where rare species may influence dissimilarity. Such differences may be due to properties of each location. Mahahual, unlike Xahuayxol, is under tourist influence and has freshwater inflow (during the rainy season) and nutrient loading.

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COORDINATED GENE TRANSCRIPTION FOR ARACHIDONIC ACID BIOSYNTHESIS IN MYRMECIA INCISA ADAPTING TO A NITROGEN STARVATION/REPLENISHMENT SHIFT

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Myrmecia incisa was characterized by a high content of arachidonic acid (ArA), especially when grown under the nitrogen starvation conditions. ArA synthesis in this alga was suggested from oleic acid via linoleic acid, g-linolenic acid (GLA) and 20:3w6 on the basis of these fatty acid changes. Accordingly,

D12, D6, D5 and W3 fatty acid desaturases (FADs) and D6 fatty acid elongase (FAE) played an important role for ArA biosynthesis. The cDNA of these genes, therefore, were cloned, and compared with their corresponding DNA sequences. All of these genes were separated by several introns. Transcription of these genes of *M. incisa* as estimated by Q-RT-PCR gradually increased during the course of nitrogen starvation, and dramatically declined from the onset of nitrogen replenishment. The percentage of GLA, 20:3W6 and ArA, which were catalyzed by D6 FAD, D6 FAE and D5 FAD, respectively, were first increased and subsequently decreased accompanying with the corresponding gene transcription. The percentage of linoleic acid, however, showed a quite contrary change to ArA, suggesting that the increased ArA in this alga during the course of nitrogen starvation possibly resulted from a reduced linoleic acid and that D6 FAD and D6 FAE were crucial enzymes for ArA synthesis. Transcription of W3 FAD gene and percentage of ALA which was catalyzed by W3 FAD showed a significant decrease under the nitrogen starvation stress followed by a sharp increase after supplement of nitrogen. The percentage (not more than 2% of total fatty acids) of EPA in this alga varied insignificantly during the process of nitrogen treatment which couldn't keep pace with W3 FAD gene transcription, suggesting that the encoded protein by the cloned W3 FAD gene from this alga might not be as a catalyst to synthesize EPA from ArA. It was concluded that nitrogen was a key factor to regulate the gene transcription cooperatively for ArA biosynthesis in *M. incisa* to adapt itself to a nitrogen starvation/replenishment shift.

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PHYLOGEOGRAPHY OF *BATRACHOSPERMUM HELMINTHOSUM* (RHODOPHYTA, BATRACHOSPERMALES) IN BRAZIL

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Batrachospermum helminthosum Bory is a widespread species of Batrachospermales in Brazil. Genetic variation among and within populations of this species was investigated using two mitochondrial markers – the *cox2-3* spacer region and the barcode region of the

gene *cox1*. 82 individuals from 11 localities were sampled from the southernmost state (Rio Grande do Sul) through Espírito Santo in southeastern Brazil (20°24'49"-29°10'44"S). The species showed a greater genetic variation among than within populations, a typical pattern for members of the Batrachospermales. Nine haplotypes were found for *cox2*, with 376 base pairs (bp) in length, which varied from 1 to 9 bp (0.3-2.4%). 10 haplotypes of *cox1* were found, with a divergence of 3 to 21 bp (0.4 to 2.8%) among them. For *cox2-3*, populations had 1 or 2 haplotypes, with 1 bp variation among them. For *cox1*, all populations had only one haplotype. For both markers an essentially similar pattern of variation was found, with a trend from the south towards the southeast regions.

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ALGAL APPLICATION IN PHYCOREMEDIATION PROCESS WITH SPECIAL REFERENCES TO ALGAE- METAL INTERACTION

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Algae have great potential as eco-toxicological test systems. In the present topic use of local algal genera for toxic and precious metal separation and their changes at cellular level would be discussed. The study would include long and short term effect of Pb and As toxicity on population pattern, reproductive process and cellular biochemistry of different algal genera and their role in nano-metal production. In natural habitat, changes in algal flora were observed due to long exposure of Pb or arsenic. Remarkable changes in reproductive behavior of eukaryotic algae were noticed such as excessive zoosporogenesis and resting spore production by *Rhizoclonium* and *Navicula* and the production of hormogonia by cyanobacterial taxa at low concentration of metals. Atomic absorption spectrophotometry and neutron activation techniques were employed to estimate the metal absorption. *Phormidium* and *Lyngbya* were further used as biofilter in immobilized condition after being bio-engineered as algae based filter for lead and arsenic removal.

DEVELOPMENT OF MICROSATELLITE LOCI FOR *PHYMATOLITHON CALCAREUM* (CORALLINALES, RHODOPHYTA) IN THE EUROPEAN ATLANTIC USING NGS TECHNOLOGY

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Maerl beds are subtidal habitats with a worldwide distribution, made up of unattached, non-geniculate coralline algae. They are considered habitats of ecological and economic importance because they harbor high biodiversity and benefit commercial fisheries. The EU Habitats Directive partially protect these beds, and its annex V lists *Phymatolithon calcareum*, a major maerl-forming coralline, as a species of community interest whose taking in the wild and exploitation may be subject to management measures. At present, however, there is an important gap in our knowledge of this rhodophyte since no study has ever elucidated its genetic diversity and population structure. Microsatellites are powerful codominant markers, particularly indicated for population genetics studies. Here, we describe the development of 11 polymorphic microsatellites loci for *P. calcareum* using 454 next-generation sequencing (NGS) technology. Two of the 11 loci showed low polymorphism but the remaining 9 seem promising for future investigations of the population structure and diploid/haploid ratios of this maerl-forming coralline. To our knowledge, this is the first time that microsatellite markers have been developed for coralline algae.

ALGAL CHEMICAL ECOLOGY IN A CHANGING OCEAN

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Increases in atmospheric carbon dioxide concentrations are reducing ocean pH and calcification rates of marine organisms, and may also cause sublethal stress and changes in concentrations of secondary metabolites that protect macroalgae from herbivores. We exposed a calcareous, terpene-producing macroalga, *Halimeda opuntia*, to seawater conditioned with increasing levels of CO₂ to test for effects of ocean acidification on growth, calcification and terpene concentrations. Although terpene concentrations were not affected, calcification decreased at CO₂ concentrations of 2200 ppm and above, which could impact herbivory on this alga. We also explored the effects of reduced seawater pH on the benthic cyanobacterium, *Lyngbya cf. confervoides*, and found no effect on growth rates over the range of CO₂ concentrations tested.

READING IN THE GENOMES OF *CHROMERA* AND *VITRELLA* – PHOTOSYNTHETIC BROTHERS OF APICOMPLEXAN PARASITES

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The chromerids are a recently discovered group of algae that live in association with corals. So far, only two distantly related species, *Chromera velia* and *Vitrella brassicaformis*, are known. Robust phylogenomic and ultrastructural evidence confirms their close relationships with the apicomplexans. Combined with their epi/symbiotic lifestyle, the available data suggest the chromerids represent a missing link in transition of free-living algae into one of the most diverse and successful groups of parasites. Analysis of organellar and nuclear genomes of *C. velia* and *V. brassicaformis* reveal several unique features. In agreement with the Chromalveolate hypothesis, plastids of chromerids are of red algal origin and represent important link between non-photosynthetic apicoplast of apicomplexans and peridinin plastid of dinoflagellates. Moreover, *C. velia* possesses the most divergent and gene-poor functional photosynthetic plastid known to date, which uses non-canonical (UGA) code for tryptophan. The peculiar life-history of chromerids is reflected in numbers of unique

metabolic traits, e.g. in synthesis of tetrapyrroles from glycine (as the only photosynthetic eukaryotes) or utilizing ornithine-urea cycle as a key compound of inorganic carbon and nitrogen metabolism.

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SEX-BIAS IN FUCOID ALGAL TRANSCRIPTOMES

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Sexual dimorphism between males and females occurs against a virtually identical genomic background. Transcriptomic datasets have recently shown extensive, genome-wide sex-biased gene expression (SBG) that presumably underlies sexual dimorphism, mainly in metazoans. The brown algae show extensive variation in life-history and reproductive system, the genomic basis of which remains largely unexplored. We recently identified SBG in male and female reproductive tissue transcriptomes in *Fucus vesiculosus*, using 454 sequencing. Here we report the results of a more extensive Illumina RNA-seq dataset. A *de novo* *F. vesiculosus* reference transcriptome was assembled from ca. 70 M 100bp paired-end reads. We identified >8,700 genes orthologous to *Ectocarpus siliculosus* (54% of the proteome), which were on average >80% full length. Differential expression (DE) analysis using biological replicates revealed extensive gender variation. The number of SBG was higher in male (2,388 transcripts) than female tissue (1,478 transcripts), a pattern commonly seen in evolutionarily distant animal models where males are the heterogametic sex. Major female SBG were carbohydrate-modifying enzymes with likely roles in zygote cell wall biogenesis and/or modification. Male SBG function in sperm development, signal perception (e.g., phototropin), transduction (several kinases), and putatively flagella-localized proteins (including candidate gamete-recognition proteins). Overall, our results suggest some constraint on female SBG (possible pleiotropy), and less constraint on males associated with sperm-specific functions. We report preliminary results from comparative analysis of RNA-seq data from other *Fucus* spp. and members of the Fucaceae.

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ANALYSES OF THE MITOCHONDRIAL AND PLASTID GENOMES IN *TOLYPELLA* A. BRAUN (CHARACEAE, CHAROPHYCEAE)

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Tolypella is the third largest genus in Characeae, an evolutionarily important lineage of freshwater green algae with an uncertain phylogenetic placement in the Charophyta. Organellar genome analyses have recovered Characeae as sister to land plants or to a larger clade that includes other charophycean algae plus land plants. However, organellar genome sequences of Characeae are only available for the mitochondrial and plastid genomes of *Chara vulgaris* and the mitochondrial genome of *Nitella hyalina*. We completely sequenced organellar genomes from 14 *Tolypella* taxa representing both sections, *Rothia* (n=8) and *Tolypella* (n=6). The mitochondrial genome sequences and lengths are remarkably conserved with a mean base-pair composition of 77,687±430 (1 SD). In contrast, plastome sequences vary among all taxa while lengths differ mainly between each section (153,438±1817 in section *Rothia* and 129,547±404 in section *Tolypella*). A comparison of genome architecture and gene-based phylogenetic relationships to better understand *Tolypella* systematics and genome evolution within Characeae will be discussed.

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MICROALGAE FOR BIOREMEDIATION OF PALM OIL MILL EFFLUENT AND PRODUCTION OF LIPID-RICH BIOMASS FOR BIOFUEL

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The palm oil industry in Malaysia generates large amounts of wastewater known as Palm Oil Mill

Effluent (POME). POME is highly organic, with high nitrogen and phosphorus contents. Treatment is via anaerobic digestion of POME. Twenty microalgae strains from the University Malaya Algae Culture Collection (UMACC) were screened for growth and biochemical composition. Three strains, with high specific growth rate (μ) and lipid content were selected for culture in POME and anaerobically digested effluent (AL). *Chlorella* UMACC 300 grew best in both POME and AL, with $\mu=0.27\text{ d}^{-1}$, biomass $448.00\pm 18.33\text{ mg/L}$ (day 12), $27.70\pm 0.93\%$ DW protein, $1.60\pm 0.07\%$ DW carbohydrate and $30.67\pm 1.38\%$ DW lipid, when grown in 25% AL in 2L flask cultures. *Chlorella vulgaris* UMACC 001 grew well ($\mu=0.51\text{ d}^{-1}$) in 7.5% POME in 5L airlift annular photobioreactors. In general, all three algal strains produced 30-64% SFA. About 88%, 66%, 79%, 45% and 42% reduction in COD, $\text{NH}_3\text{-H}$, $\text{PO}_3\text{-P}$, $\text{NO}_3\text{-N}$ and $\text{NO}_2\text{-N}$, respectively were achieved. Results show that *Chlorella* UMACC 300 has potential for bioremediation of POME and production of biodiesel.

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COMPARATIVE GENOMICS OF HETEROKONT ORGANELLES

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Heterokonts comprise some of the most productive and biologically diverse lineages in marine ecosystems. Members of these lineages generate approximately half the world's oxygen. However, we know little about their organellar genomes. A project was initiated to understand the organellar genomes of these important primary producers. To assure the isolation of pure genomic DNA cultures were grown with GeO_2 to inhibit diatom growth before DNA extraction. Data from a single HiSeq lane were collected and assembled. We report here on the size, structure, and gene content of selected heterokont lineages that are closely related to or members of the brown algae (Phaeophyceae).

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DIVERSITY AND DESICCATION TOLERANCE OF ZYGNEMA

(ZYGNEMATOPHYCEAE, STREPTOPHYTA) ON SVALBARD (HIGH ARCTIC)

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Zygnema sp. is one of the most common streptophyte algae in the Arctic. It forms extensive mats in shallow freshwater pools where algae are subjected to various stress factors including freezing, UV radiation and desiccation. Although production of specialized cells (zygospores, parthenospores and aplanospores) has been reported in *Zygnema*, we did not observe their formation in natural conditions. Only senescent cells called pre-akinetes and akinetes were recorded in desiccated sites and during winter. Several *Zygnema* strains were isolated from mats into cultures for further molecular and ecophysiological characterisation. Based on *rbcL* sequences six different genotypes of *Zygnema* were detected altogether that fall into both major lineages within the genus. Moreover, filaments of *Zygnemopsis* were co-occurring within *Zygnema* mats. The desiccation tolerance of the isolated strains was investigated in experimental conditions. It was found that the survival capacity was influenced by nutrient supply, desiccation rate and age of the cultures; starved cultures showed higher resistance. *Zygnema* on Svalbard is in general resistant to desiccation stress, but strain-specific characteristics were revealed which shows their different ecological strategies within the mats.

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DIATOMS FROM THE GENUS NITZSCHIA FOR LARGE-SCALE CULTIVATION FOR LIPID AND OMEGA-3 FATTY ACIDS (EPA AND DHA)

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Thirteen diatom strains of *Nitzschia* were isolated from California and Hawaii and then screened for their feasibility for photosynthetic lipid production and

cultivation in large open-pond systems. Strains were screened at different temperatures and nutrient concentrations in the laboratory for their growth, lipid production, and EPA and DHA content. *Nitzschia* EPA and DHA content, expressed in terms of percentage of the total lipid content, ranged from 3 to 38% and from 0 to 3% respectively. The fraction of EPA and DHA in the total lipid increased with decreasing overall lipid content. High concentrations of nitrogen and silica in the media resulted in lower overall lipid content, but contributed to higher EPA and DHA fractions in the lipid. Further, *Nitzschia* cultivated in lower temperatures had higher EPA and DHA fractions in the extracted lipid relative to higher temperature growth conditions. *Nitzschia* was successfully cultivated in an open-pond system and had productivities comparable to green algae. Species from genus *Nitzschia* are therefore good candidates for lipid production for fuel and omega-3 fatty acids.

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MOESTRUPIA OBLONGA (DINOPHYCEAE) MAY CONTAIN SEVERAL CRYPTIC SPECIES

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Several dinoflagellate strains were successfully isolated from marine samples taken from various shore locations in Okinawa Island, Japan. The strains shared several common characters, e.g., the cells were unarmored and rice-grain like in shape, with a conspicuous large pyrenoid located in the middle of the body while the nucleus was positioned in the hypocone, cingulum displacement was about one third of the body length and the chloroplasts were somehow reticulated. Based on the morphological characteristics and our molecular phylogenetic analyses based on SSU and LSU rDNA, we identified the strains as *Moestrupia oblonga* (syn.: *Gyrodinium oblongum*). However more than 5 bp substitutions were found among the sequenced strains and detailed ultrastructure observation revealed that one or more of our strains had an epicone size that was relatively larger than the hypocone. Cell size differences were also observed (e.g., *Moestrupia oblonga*: 13-17 µm

long; B74: 12-19 µm long; Gyob8: 17-25 µm long; TM24: 18-22 µm long; HG151: 17-24 µm long, etc.). Based on the phylogenetic analyses and morphological observation, we suggest that *Moestrupia oblonga* may contain several cryptic species.

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MORPHOLOGICAL DIVERSITY OF THE DIATOM GENUS *MINIDISCUS* HASLE (THALASSIOSIRALES, BACILLARIOPHYTA) IN THE ATLANTIC AND GULF COASTS OF FLORIDA, USA

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Phytoplankton samples from the coastal regions of northwest and northeast Florida contain a remarkable diversity of forms from the diatom genus *Minidiscus* Hasle (Thalassiosirales). Currently at least six species are recognized in the genus; additional forms have been noted, but not in sufficient numbers to permit the formal description of new taxa. Several of the known species are frequent members of the nanoplankton of the Gulf and Atlantic coasts of Florida, including *M. trioculatus* (Taylor) Hasle, *M. chilensis* Rivera and *M. comicus* Takano. Here we document three additional morphotypes, herein referred to by their primary collection sites: the Panacea morphotype, the St. Andrew morphotype, and the Apalachee morphotype. All three are similar to *M. comicus* in the absence of a marginal hyaline area and in the presence of fultoportula surrounded by three satellite pores. The Panacea & St. Andrew morphotypes were abundant in collections from Apalachicola Bay and Rookery Bay, Florida, and from the coast of Massachusetts, suggesting both are widely distributed. The Apalachee morphotype has only been observed in the Econfina and Fenholloway estuaries of Apalachee Bay. The Panacea morphotype is characterized by prominent, evenly spaced, turbinate external tubes of the 3-5 fultoportulae and by the presence of a single row of pores, 3 in 10 µm, in an otherwise hyaline marginal area. The St. Andrew morphotype is distinguished by extremely small cells, 1.5-4.4 µm in diameter, a deep mantle, fultoportulae with simple external tubes, and

by scapulate extensions of the areolar walls. The Apalachee morphotype has denser areolae than other two morphotypes. We believe these differences provide sufficient basis for describing each of the morphotypes as a distinct species. Our observations suggest that members of *Minidiscus* may represent an important source of primary production easily overlooked due to their small size.

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HIDDEN DIVERSITY OF TERRESTRIAL GREEN ALGAE (VIRIDIPLANTAE) DISCOVERED BY ITS-2 DNA BARCODE

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Traditionally terrestrial green algae have been classified according to the morphological species concept. However, phylogenetic analyses of SSU rDNA sequences have revealed that most of the traditional genera and species are polyphyletic and need taxonomic revisions. Many terrestrial green algae show a phenotypic plasticity demonstrated by experiments of cultured material. This makes the unique species identification very difficult. In addition, many microalgae show only few diacritical morphological characters. Unfortunately most of the terrestrial green algae reproduce only asexually and the biological species concept cannot be used for identification. The newly developed ITS-2 DNA barcode correlated with the biological species concept and has been now extrapolated to asexual species and genera. Using this barcode terrestrial green algae could be clearly identified at species level and new lineages could be discovered. The new species have been confirmed using an integrative approach, which includes phylogenetic analyses of SSU and ITS rDNA sequences with morphology and life cycle of these species as well as physiological and biochemical characters (i.e. polyol production). Based on these results traditional terrestrial genera such as *Chlorella*, *Coccomyxa*, and *Chlamydomonas* have been revised and new genera and species have been described. Unexpectedly many lineages contain several isolates, which either morphologically develop a high degree of phenotypic plasticity, but showed a low genetic diversity like in *Chloroidium* and *Coccomyxa*, or, strains with morphological unity have a high genetic

variability like in *Jaagichlorella*. Summarizing, the ITS-2 barcode can be used to identify species and discovered hidden genetic diversity.

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ALGAE ON THE ROCKS: ADAPTATION THROUGH HORIZONTAL GENE TRANSFER IN THE CRYPTOENDOLITHIC RED ALGA *GALDIERIA PHLEGREA*

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Thriving in the hot, acidic, and metal-rich environments associated with geothermal areas is possible for only a few eukaryotes, with the Cyanidiophytina red algae (*Cyanidium*, *Galdieria*, and *Cyanidioschyzon*) being one of the most famous examples. These unicellular taxa can live in pH 0-4 and temperatures reaching 56°C. Because Cyanidiophytina is sister to a vast array of mesophilic red algae (the Rhodophytina) such as the unicellular *Porphyridium* and the seaweed *Chondrus*, the genetic basis of their adaptation to extreme environments is of high interest from both the perspective of biotechnology and of evolution. The recently completed 13.7 Mbp genome sequence from the hot-spring dwelling *Galdieria sulphuraria* demonstrated that horizontal gene transfer (HGT) from prokaryotic sources has provided this taxon with remarkable metabolic versatility (e.g., glycerol metabolism) and the ability to survive in its hostile environment (e.g., genes to detoxify mercury and arsenic). To explore the role of HGT in other members of this genus, we generated an 11.4 Mbp draft genome assembly from the sister taxon *G. phlegrea* DBV 009. In contrast to *G. sulphuraria* this species is adapted to dry habitats near fumaroles such as fissures between rocks or cryptoendolithic environments. Here, we provide evidence of extensive gene loss in the common ancestor of Cyanidiophytina that includes the eukaryote-derived loci required for urea utilization. Surprisingly, we find that *G. phlegrea* has regained the complete set of genes required for urea hydrolysis through HGT from eubacteria. This acquisition presumably reflects adaptation to the drier endolithic

environment where nitrogen may be limiting. Our study demonstrates that genome reduction, which is a common outcome of adaptation to an extreme (or specialized) niche in eukaryotes, can be ameliorated by the gain of once lost, or novel functions via HGT.

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IDEALG PROJECT: 10 YEARS TO MAKE THE BEST OF ALGAL GENOMICS AND POST-GENOMICS FOR THE DEVELOPMENT OF SEAWEED AQUACULTURE AND ASSOCIATED BIOTECHNOLOGIES

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In response to the constantly increasing demand for seaweed biomass on an international level, the need to upscale or develop cultivation methods of native seaweeds in Europe is becoming urgent. The French large integrated long-term research project “IDEALG” proposes to tackle issues related to seaweed aquaculture and biomass exploitation. Special attention will also be addressed to economic, social and environmental impacts of such developments on other maritime activities, conservation and bio-safety issues. IDEALG project will help improve algal bioresources and seaweed domestication as well as boost seaweed biotechnologies by making the best of genomics and post-genomic research. Indeed, metagenomic approaches applied on seaweed biomass and closely associated micro-organisms will bring progress to seaweed crops and developing blue and white technologies. First results have already brought light on strategic species to study, on algae genotyping, on nutritional characteristics of local seaweed and on green algae aquaculture. Social and economic aspects of the seaweed industry in France have also been reported but will continue to guide IDEALG along strategic decisions in order to ensure a sustainable development.

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EFFECT OF TEMPERATURE FLUCTUATIONS ON MICROALGAE GROWTH AND CONTENT: A REVIEW

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Temperature plays a crucial role in microalgae productivity, although existing studies have considered that light was the main productivity driver. High rate outdoor production units of microalgae can undergo large temperature fluctuations which can exceed optimal conditions. Temperatures exceeding optimal growth temperatures of microalgae are more deleterious than lower temperatures and can hence deeply affect productivity. The objective of the presentation is to review the effects of temperatures, and especially high temperature, on microalgae growth and intracellular content. The effect of high temperatures is clearly species dependent. Moreover, long term adaptation of certain species over generation cycles has also been proven efficient to increase optimal temperatures. Temperature can also drive chlorophyll or lipid contents. Physical models coupled to biological kinetics are able to predict the evolution of temperature in the growth media and its effect on the growth rate, highlighting the downstream drastic economic and environmental impacts. These elements can complement existing models and help visualize the effective impacts of temperature on outdoor cultures.

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AN ULTRASTRUCTURAL STUDY OF GAMETOGENESIS IN THE ARAPHID PENNATE DIATOM *PESUDOSTAurosira TRAINORII*

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Pseudostaurosira trainorii, an araphid pennate diatom, has anisogamous sexual reproduction, which shows a number of unique features. The ultrastructure (TEM) of gametogenesis is described. As gametogenesis progress the cells elongate (in girdle plane) to 8-12 μm and the nuclei increase in size (4-4.5 x 3 μm) indicative of meiosis. Following meiosis in female cells the upper valve is shed, exposing the naked binucleate female gamete that slides out of the valve and

becomes more rounded. The male gamete appear to contain a single functional (2.0 x 1.5 µm) nucleus and a second smaller (1.5 x 1.0 µm) “nucleus-like body”, that has dense membranes and is presumed to be a degenerating nucleus. The fine protoplasmic threads associated with the motile male sperm could be protoplasmic extrusions as there is no evidence of kinetosomes associated with sperm nuclei. Following the mixing of the gametes, elongate auxospores develop. They have a centrally located nucleus that is surrounded by a halo of Golgi dictyosomes. The ultrastructural changes discussed in relation to the known sexual cycles of diatoms.

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EVIDENCE FOR ABC PROTEINS ACTIVITY IN SECONDARY METABOLITES TRANSPORT AND CHEMICAL DEFENSE IN RED SEAWEED *LAURENCIA DENDROIDEA*

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The intracellular transport of secondary metabolites in red algae is not well known, including their transport to storage organelles, such as the *corps en cerise* (CC) found in *Laurencia dendroidea*. Our goal was to verify the presence and activity of ABC proteins transporting secondary metabolites in *L. dendroidea*. To avail ABC activity, algae were incubated with Rho123, a fluorescent substrate of ABC proteins, in the presence/absence of Cyclosporine (CSA), an inhibitor of ABC activity. Ten days long assays were performed to avail the effects of ABC inhibition on cell morphology, secondary metabolites storage and fouling defense. Analysis of cDNA expression was also performed to identify ABC related genes. Results revealed the presence of ABC transporters in membranes of CC, once Rho123 internalization was not observed after CSA incubation. The ABC inhibition also induced changing of CC morphology, decreasing of stored secondary metabolites and reduction of fouling defense. Eight ABC cDNA sequences similar to secondary metabolites

transporters found in plants were identified. Concluding, we suggest that ABC proteins are related to secondary metabolites transport and chemical defense in *L. dendroidea*.

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DNA SEQUENCING PROVIDES NEW INSIGHTS INTO THE DIVERSITY OF NONGENICULATE CORALLINE ALGAE (CORALLINALES, RHODOPHYTA) IN THE NORTHWESTERN GULF OF MEXICO

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The 2010 Deepwater Horizon crude oil spill in the Northwestern Gulf of Mexico is ostensibly correlated with a decline in species richness and abundance of macroalgae growing in subtidal hard bank communities offshore Louisiana and Texas at 45-90 m depth. Nongeniculate corallines were among the few visible seaweed species in the oil-impacted areas, and results from five post-spill dredging surveys indicate that both rhodolith forming corallines and nongeniculate corallines epiphytic on rhodoliths survived the oil spill insult although the number of species and their biomass in the field was, and still is, dramatically reduced. Comparative phylogenetic analyses of plastid (*rbcL*, *psbA*), nuclear (*LSU*), and mitochondrial (*COI*) loci of nongeniculate corallines collected pre- and post-spill reveal numerous taxa spanning multiple lineages for the region. Identification of these Corallinales at the species level is not yet possible because available names need to be validated by comparisons to type specimens. Nine species are reported, including *Lithothamnion* spp. and *Mesophyllum* spp. *sensu lato* (Melobesiaceae, Hapalidaceae), *Lithophyllum* sp. (Lithophylloideae, Corallinaceae) and *Porolithon* spp. (Porolithoideae, Corallinaceae).

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A MASSIVE AND UNUSUAL DIATOM BLOOM: ECOLOGY AND OCEANOGRAPHY OF A RARELY REPORTED PLANKTONIC *HASLEA* IN EAST SOUND, WASHINGTON, USA

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Seasonal blooms of planktonic diatoms are common in coastal and upwelling regions of the oceans. Although many blooms are highly diverse, some are dominated by one or a few taxa, generally a common diatom well-known for its ability to achieve high population density. It is far more unusual to encounter a massive bloom of a rarely reported diatom. During an interdisciplinary oceanographic field program, we encountered a massive, nearly monospecific bloom of a planktonic *Haslea*, and tracked the population over space and time using scanning flow cytometry, microscopy, in-situ optics and airborne lidar. Early in the experiment, this diatom formed intense, buoyant surface slicks and thin layers, with concentrations as high as ~5500 cells/mL. One week later, highly pigmented, live cells were located in a broad peak at mid depth, while those in overlying waters were mostly dead. When the entire population finally crashed, dead cells floated instead of sinking, forming thick clots on the surface. The evolution of this unusual bloom will be discussed in light of the potential relevance of allelopathy, suppressed grazing pressure, viruses and lipid formation.

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SOFT-ALGAE AS WATER QUALITY INDICATORS – AN INVESTIGATION USING THE 2008-2009 EPA NATIONAL RIVERS AND STREAMS ASSESSMENT (NRSA)

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Where diatoms are regularly used for water quality assessment the other species of algae present in rivers and streams, often termed soft-algae, are used relatively little. Using one of the exceptions, Potapova's work in 2005 with the USGS National Water-Quality Assessment (NAWQA) data, as a starting point this investigation examines whether there are reliable indicator species within the soft-algae assemblage using current protocols. Results

showed possible preferences at the higher taxonomic levels of Phylum, Order or Family. Some species were identified as possible indicators for particular water quality issues, but further research will be required. Overall the conclusions indicate that data collected from samples analyzed using current protocols were too weak to identify soft-algal indicators and revision is required.

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PHYLOGENETIC TAXONOMY OF *COLPOMENIA* (SYCTOSIPHONACEAE, PHAEOPHYCEAE) FROM THE GULF OF CALIFORNIA

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Colpomenia is one of the main biomass producers along the Gulf of California. Historically 4 specific names have been used for the area and a potential 5th species has been suggested recently. We developed an intensive sampling around the Gulf of California and based on morphological, anatomical and molecular data have reached the conclusion that 5 species are present in the area included a new species in the genus. *Colpomenia duvilleae* is confirmed in the area and *C. paheodactylia* is no longer recognized for the Gulf of California. There are 3 widely distributed species and 2 endemic to the region, the last 2 are proposed to be considered as species under special consideration in the Mexican Environmental Norm.

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INFLUENCE OF SOUTH ATLANTIC CENTRAL WATERS IN MACROALGAL RICHNESS

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A recent survey performed in a Marine Park of São Paulo state, Brazil (Laje de Santos Marine State Park), reported macroalgal richness significantly higher in summer/fall than winter/springs and hypothesized that this may be the result of presence/absence of water mass: South Atlantic Central Water (ACAS). To test

this hypothesis, samples were collected in the other three insular conservation units along São Paulo state, in two different seasons: summer/fall (ACAS is present) and winter/springs (ACAS is absent). Samples were collected in the intertidal and subtidal zones (depths of 5, 10 and 15 m). Materials for morphological study were fixed in 4% formalin/seawater. A PCA analysis was performed to correlate the variation of macroalgal richness to the seasons. The results identified 112 taxa, showed that the species richness is highly correlated with season (higher in summer/fall) and presented a significant variation in richness (32-56% higher in summer/fall). This could be result of ACAS presence that thermally stratifies the environment and increases the nutrient availability to the ecosystem. More studies are necessary to check if other factors have important role on these results.

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TISSUE FUSION BETWEEN *HYPNEA PANNOSA* AND *H. SPINELLA* (CYSTOCLONIACEAE, RHODOPHYTA) PATCHES: A MORPHOLOGICAL DESCRIPTION

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Hypnea pannosa and *H. spinella* (Cystocloniaceae, Rhodophyta) are the two most common species in the intertidal rocky shores at the Mexican tropical Pacific coast. These species grow in environments with significant variations in light and water motion regimes. Each species' patches usually grow in different morphs, the first as prostrate cushions, the second as erect tufts. Under some particular environmental conditions, these two species grow in adjacent patches and are often physically linked, forming morphologically mixed patches. This situation caused taxonomic confusion for a long time because this third morph looked like a different species; the issue has now been resolved. Microscopic analyses allow us to reconstruct of the fusion process between the branches of both species that are *a posteriori* event producing a particular cytological relationship. In this study we describe and illustrate the tissue fusion process occurring between these two species, and compare them with the coalescence process described

for other red algae, defining similarities and differences between both tissue processes.

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***PTILOCLADIOPSIS HORRIDA*, A RARE SPECIES FROM THE MEDITERRANEAN SEA, BELONGS TO AN UNDESCRIBED BASAL FAMILY IN THE DUMONTIOID LINE (GIGARTINALES, RHODOPHYTA)**

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Ptilocladopsis horrida was originally described from the Bay of Naples, Italy, by Berthold. Most authorities have followed Berthold (1882), Schmitz (1889) and Feldmann-Mazoyer (1941) in placing it in the order Ceramiales. The thallus is initially filamentous, becoming compressed, with four periaxial cells per axial cell that elongate and flatten at their tips leaving a hollow space. Cells of the filaments transform progressively into a pseudoparenchymatous cortex leaving a layer of spiniform filaments covering the thallus surface. Plants are monoecious and tetrasporophytes are unknown. Carpogonial branches form on unmodified filaments and are four-celled and strongly recurved. Fertilized carpogonia fuse with the third cell of the carpogonial branch and produce 1-4 branched connecting filaments. Cells of remote vegetative filaments as well as the supporting cell of the carpogonial branch may function as auxiliary cells. An involucrate gonimoblast is produced from a single primary gonimoblast cell that fuses with the diploidized auxiliary cell, and bears carposporangia in chains. Preliminary molecular evidence places *Ptilocladopsis* in a sister relationship to basal members of the Gigartinales.

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PHYTOBENTHIC COMMUNITY DEVELOPMENT UNDER STATIC AND DIURNALLY OSCILLATING PH: A CASE STUDY IN THE SOUTHERN TEMPERATE OCEAN

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Community development under static and metabolically-controlled pH fluctuation under present (mean pH= 8.05) and future acidified ocean (mean pH= 7.65) scenarios was investigated in the laboratory. After 5 months, phyto-benthic community composition was measured. Different species of benthic diatoms (*Navicula*, *Cocconeis*, *Nitzschia*, *Fallacia*, *Achnanthes* and *Cylindrotheca*) and juvenile fleshy (e.g. *Durvillaea* sp., *Desmarestia lingulata*, *Dictyota* sp., turf brown and greens, and foliose and filamentous reds) and coralline macroalgae were observed. Growth of crusts of the coralline *Arthrocardia corymbosa* was 37-56% lower under the fluctuating pH in an acidified ocean scenario relative to the other static and fluctuating pH treatments, while the number of growing upright fronds was highest under the present day fluctuating pH scenario. The %MgCO₃ in the calcite of juvenile coralline recruits was reduced by 14.5% under future low pH scenarios. The chl *a*, biogenic silica, and C:N:P ratios of the diatom assemblage did not differ between treatments. Community metabolism modified seawater carbonate chemistry during the 4 between seawater replenishment within the culture chambers, and extremely low night-time pH was responsible for the negative effects on pH-sensitive species.

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HOW CAN DINOFLAGELLATES HELP IN REDUCING CORAL REEF VULNERABILITY TO ENVIRONMENTAL STRESS?

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Reef-building corals form mutualistic symbioses with unicellular photosynthetic dinoflagellate of the genus *Symbiodinium*. Exposure to ultraviolet radiation especially when combined with thermal stress, has been recognized as an important abiotic factor leading to oxidative stress and break down of the coral-algal endosymbiosis. In nature, many marine organisms use Mycosporine-like Amino Acids (MAAs) as biological sunscreens in UV protection and the prevention of oxidative stress. Corals acquire MAAs from their

symbiotic algae and diet. Higher diversity of MAAs discovered within the coral host compared to their algal endosymbionts may be due to a regulation of MAA synthesis by host factors and/or due to host heterotrophy. The biosynthesis of MAAs has been proposed to occur via either the shikimate and/or pentose phosphate pathways. Until now the complete enzymatic pathway of MAA synthesis is not known nor is the extent of their regulation by environmental conditions. Here, using a transcriptome mining approach, we distinguish the gene homologs from the shikimate and pentose phosphate pathway involved in MAA biosynthesis within the sequences of coral dinoflagellates. We also describe the highly similar sequences of genes from the proposed MAA biosynthetic pathway involved in the metabolism of 4-deoxygadusol (direct MAA precursor) in different *Symbiodinium* strains confirming their algal origin and evolutionary conserved nature. Finally, we unveil the separate identity of 4 genes from the proposed MAA (shinorine) biosynthetic gene cluster in symbiotic dinoflagellates. This work provides a novel phylogenetic/biochemical outline of the genes involved in MAA biosynthesis in coral dinoflagellates. Consequently, studying the pathways that strengthen the ability of algae, corals and other marine organisms to avoid stress (such as the MAA photo-protective role) is fundamentally important for improving our understanding of the impacts of increasing global stressors.

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EXTREMOPHILES AND THE SEARCH FOR LIFE IN THE UNIVERSE

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Each recent report of liquid water existing elsewhere in the solar system has reverberated through the international press and excited the imagination of humankind. Why? Because in the last few decades we have come to realize that where there is liquid water on Earth, virtually no matter what the physical conditions, there is life. What we previously thought of as insurmountable physical and chemical barriers to life, we now see as yet another niche harboring “extremophiles”. This realization, coupled with new data on potentially habitable moons and planets, the survival of microbes in the space environment and

modeling of the potential for transfer of life between celestial bodies, suggests that life could be more common than previously thought. Here we critically examine what it means to be an extremophile, the implications of this for the search for life in the cosmos.

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ANALYZING THE EUGLENOID PLASTID GENOME

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Euglenoids are unicellular asexual flagellates with photosynthetic members due to secondary endosymbiosis. The aim of this research was to sequence the chloroplast genome of *Euglena clara* and compare it to other euglenoid chloroplast genomes, in particular other members of the genus *Euglena*, *E. gracilis*, *E. viridis* and *E. hiemalis*, to determine the variability of the chloroplast genome within a single genus. For this study, the plastid genome of *Euglena clara* was sequenced (Roche 454 and Illumina HiSeq Sequencer), assembled (CLC Genomics Workbench and Newbler) and annotated (BLAST and Dual Organellar Genome Annotator (DOGMA)). Based on our initial analysis, the genome of *E. clara* may have the smallest chloroplast genome in the genus *Euglena* by as much as 20 kb, while maintaining all of the core chloroplast genes. The reduction in size is due to a combination of fewer and smaller introns, compared to other members of this genus.

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CELLULAR PROCESSES RELATED TO CHEMICAL DEFENSE AND TO SYNTHESIS, STORAGE AND TRAFFIC OF SECONDARY METABOLITES IN LAURENCIA COMPLEX

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The *Laurencia* complex comprehends many species producing a variety of molecules with ecological and biotechnological importance. Thus, we have been studying these algae and their cellular/molecular processes to better understand the synthesis, storage and traffic of secondary metabolites. New results revealed that, despite the variation of cell morphology of species, the storage of secondary metabolites occur in specific and specialized vacuoles derived from Golgi apparatus. These metabolites can be synthesized by two different biochemical pathways, mevalonate and non-mevalonate (once both occur in a same species). The intracellular traffic of secondary metabolites occurs within vesicles but also through membranes (depending on ABC proteins). The vesicle traffic depends on complex cytoskeleton involvement and, activated by environmental factors, is essential to secondary metabolites exudation to thallus surface. ABC proteins play an essential role in metabolites transference to storage organelles (to *corps en cerise*). Their inhibition leads to the impairment of seaweed chemical defense, resulting in growth of fouling organisms over algae surface. Finally, we obtained results that reveal the presence of lipids on algae surface and their activity against fouling organisms.

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MORPHOLOGIES AND PHYLOGENETIC CHARACTERISTICS OF TWO NOVEL DINOFLAGELLATES WITH GREEN-COLORED CHLOROPLASTS

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We recently isolated novel green-colored dinoflagellates from sea water sampled in Japan, and successfully established two strains (TGD and MGD). Importantly, they are morphologically distinctive from the previously studied green-colored lineage *Lepidodinium* spp. Strains TGD and MGD have been stably maintained as mono-algal cultures in the laboratory for more than a year. Both strains are ellipsoidal cells, measuring ca. 15.4 µm long and 8.7 µm wide, and ca. 14.9 µm long and 7.9 µm wide, respectively. The two strains are different from each

other in the following points: the shape of the posterior end of the hypocone, the size of the apical groove, the overall shape and number of chloroplasts, and the arrangement of pyrenoids. Interestingly, the periplastidal compartment with ribosome-like particles was identified in both strains. We determined the host nucleus-encoded 28S and plastid-encoded 16S rDNA sequences of strains TGD and MGD. The host and plastid phylogenies suggest that MGD, TGD, and *Lepidodinium* spp. are distantly related from one another, but are common in possessing pedinophyte-derived chloroplasts. The results imply that the diversity of 'green' dinoflagellates has been largely underestimated.

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MOLECULAR DIVERSITY OF THE ENDOLITHIC SIPHONOUS GREEN ALGAL GENUS *OSTREOBIUM* (BRYOPSIDALES) IN THE RYUKYU ARCHIPELAGO, JAPAN

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Molecular characterization (plastid-encoded *tufA* gene) of *Ostreobium* spp. associated with shallow water reef-building corals across four islands of the Ryukyu archipelago revealed 20 species-level entities spread across 5 family-level lineages. The core of *Ostreobium* diversity (>15 spp.) represented a large monophyletic lineage sister to the remainder of the Bryopsidales (the Bryopsidinae and Halimedinae), while a second, less diverse lineage (5 spp.), was sister to the Rhipiliaceae (within the Halimedinae). The amount of genetic divergence in the core lineage of *Ostreobium* spp. exceeded variation found within the suborders Bryopsidinae and Halimedinae, justifying formal recognition for a separate suborder. The relationships of *Ostreobium* spp. to coral species were not mutually exclusive.

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WAVE IMPACTS, MULTIPLE STRESSORS AND FRACTURED CONNECTIVITY OF ALGAL-DOMINATED SHORES IN A CHANGING CLIMATE

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The sustainable use of marine resources depends to a great extent on the maintenance of diversity and functional relationships. In the nearshore environment, this involves habitat-forming seaweeds, particularly fucoids and kelps, which facilitate the presence of many other species and underpin primary production. Unfortunately, multiple stressors and a changing wave climate have greatly affected onshore algal-dominated communities, fracturing connectivity among populations, disturbing recruitment dynamics, and compromising their resilience. Popular paradigms of grazer dynamics, trophic cascades and implementation of protected areas do little to address the diffuse and wide impacts affecting many communities. This is discussed using examples from New Zealand and the west coast of North America.

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ENHANCEMENT OF PUFAS, PHYCOBILINS AND CAROTENOIDS IN TWO IRISH MACROALGAL SPECIES (*PALMARIA PALMATA*, *FUCUS SERRATUS*) THROUGH OPTIMISED CULTIVATION

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Seaweeds are a rich source of chemical compounds with bioactive properties, however their commercial utilisation is still hindered by a lack of understanding how to actively influence their chemical profile and potentially enhance desired compounds. In order to grow macroalgae for specific target compounds such as carotenoids, PUFAs and phycobilins, a better understanding of the effects of abiotic factors is needed. Results presented here are part of NutraMara, Ireland's Marine Functional Foods Research Initiative, which aims at an improved commercial utilisation of Irish marine resources with a specific focus on the optimised cultivation of algae. Macroalgae of commercial interest (e.g., *Palmaria palmata*, *Fucus serratus*) were exposed to multifactorial experimental conditions including different temperature, light, nutrient and carbon levels. Chemical responses in terms of levels of phycobilins, carotenoids, PUFAs

and MAA were quantified after experimental exposure. Physiological parameters and growth were also measured throughout the experiments. Preliminary results showed significant effects of culture conditions on bioactive profile. The results aim at supporting industry in the selection of culture conditions in order to apply high-value algal biomass as a source for valuable bioactive food compounds.

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THE EFFECTS OF SALINITY AND CADMIUM ON THE ARCHITECTURE AND METABOLISM OF RED ALGA *PTEROCLADIELLA CAPILLACEA*

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Over the past few years, increase of human population and industrial development has led to a rise of cadmium pollution in aquatic systems. *Pterocladia capillacea* is distributed along the Brazilian coastline and it's a well known source of agar. The aim of the study was to analyze the effects of salinity and cadmium on cellular architecture and metabolism of *P. capillacea*. The samples were collected from Armação Beach, Florianópolis. Subsequent experiments were performed using different salinities (25, 35 and 45 psu) and cadmium concentrations [0 (control), 0.17, 0.35, and 0.70 ppm], added as CdCl₂. In general, under 0.17, 0.35 and 0.70 ppm of cadmium, irregular shape of cortical cells was evident. Numerous electron-dense precipitates in the cell wall and cytoplasm were observed. Disrupted thylakoids were frequent as well as an increase of plastoglobuli. Level of ultrastructural disorganization and irregular shapes were more dramatic at higher concentrations of cadmium, corroborating the alteration on physiological responses of growth rate and photosynthetic pigments. Moreover, drastic alteration on mitochondrial organization was observed, compromising seriously primary metabolism, production of carbohydrate (photosynthesis) and energy (respiration).

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A DYNAMIC APPROACH TO THE STUDY OF RHODOLITHS: A CASE STUDY FOR THE NORTHWESTERN GULF OF MEXICO

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In the northwestern Gulf of Mexico beds of rhodoliths and unconsolidated rubble are associated with unique offshore deep bank habitats, the salt domes peculiar to that part of the northern Gulf at a depth of 45-90 m. These rhodolith habitats harbored the highest known seaweed diversity in the northern Gulf prior to the 2010 BP Deepwater Horizon oil spill disaster. Five post-spill collecting cruises to two biodiversity rich pre-oil sites off the coast of Louisiana revealed a dramatic die-off of seaweeds at those sites. During our most recent sampling in November 2012, seaweeds were significantly repressed in Fish Haven and Sackett Banks and had not recovered to pre-spill diversity and biomass. Dredged "bare" or partly algal-denuded rubble maintained as "live rocks" in 20-gallon tanks on the laboratory gradually became covered by a suite of red, green and brown seaweed germlings that to this day continue to grow to adult size, reproduce, and go through a series of temporal biomass decline and subsequent regrowth, reflecting pre-spill composition at each site. Other species currently growing and reproducing in our tanks had not been observed in the field during pre-spill sampling. Scanning electron microscopy with energy dispersive X-ray spectroscopy (SEM-EDS) for elemental analysis and chemical characterization enables us to understand the complex micro-anatomy of the nodules' surface and their internal structures and microbiota. We hypothesize the function of rhodoliths and rubble as marine seedbanks for biological diversity and explore the role of cryptic stages in overall community resilience following a major anthropogenic disaster.

A DISTINCTIVE SECOND NEW SPECIES OF *CRASSITEGULA* (RHODOPHYTA, SEBDENIALES) FROM BERMUDA BASED UPON MORPHOLOGICAL AND MOLECULAR DATA

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Since the genus *Crassitegula* was described less than a decade ago based upon a single species from Bermuda in the western Atlantic, one additional species was described with a type locality in Lord Howe Island, Australia. These two species, *C. walsinghamii* (Bermuda) and *C. imitans* (Lord Howe), are similar in habit with dorsiventrally arranged reniform, subcircular to irregular blades issuing new lobes marginally, often leading to an overlapping population of individuals. Using COI-5P sequences for barcoding of recent collections from Bermuda, a third morphologically dissimilar species in the genus appeared in the generated phylogenetic tree embedded between the two known species, with 7.4% base pair differences from the genotype, *C. walsinghamii*. Therefore, in this paper we are proposing a third species in the genus and the second from Bermuda. The new taxon has a unique habit for *Crassitegula* along with its distinctive genetic characteristics. Unlike the other two species, the new collections have deeply dissected blades and are distinctly smaller in size. Some plants are broad with finger-like projections, while others are strap-shaped and irregularly branched. Based upon this molecular and morphological evidence, we will propose this as a putative new species of *Crassitegula* highlighting its obvious dissected blades. This represents another genus with novel species in both Bermuda and Lord Howe Island along with *Asteromenia*, *Halopeltis* and *Meredithia*.

CHANGE YOU CAN BELIEVE IN: CLIMATE CHANGE IMPACTS ON ANTARCTIC MACROALGAE

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The past centuries' anthropogenic emissions have increased the amount of CO₂ absorbed into the world's oceans, altering the balance of carbonate species and lowering average ocean pH globally. Currently, climate change projections predict a decline in pH from today's average level of 8.1 to 7.7 by 2100, which will coincide with increased atmospheric and oceanic temperatures. Along the western Antarctic Peninsula (WAP) macroalgal communities can be very dense, especially above 30 m depth where percent cover of encrusting coralline algae can reach up to 77% and canopies of *Desmarestia* spp. can cover up to 100% of the benthos. The goal of this project is to investigate physiological responses of common Antarctic algae species to reduced seawater pH and rising seawater temperature. Microcosm experiments using a 2 x 2 factorial design reflecting current conditions and near-term condition predictions for pH and temperature along the WAP were set up at Palmer Station. A yet-to-be-identified crustose coralline alga, a non-calcified crustose red alga, *Hildebrandia lecanelli*, and the canopy species *D. anceps* and *D. menziesii* were maintained for ~2 months in these microcosms. Calcification of thalli, bleaching of thalli, chlorophyll a content, photosynthetic characteristics, and growth were measured to compare physiological responses between treatments. Results may indicate potential for a regime shift between calcified and non-calcified algae in the marine benthos along the WAP, as well as physiological impacts on the canopy forming species in the area.

DAWN IN THE AGE OF ROBOTIC PHYCOLOGY

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Algae play a disproportionately large role in the metabolism of the Earth and yet understanding the physical/chemical regulation of their physiology and ecology on a changing aquatic planet remains an open problem. This, in part, reflects our limited ability to sample space and time. Fortunately environmental science is in the midst of a technical revolution in both

the sensors available to the community and the robotic systems that are capable of carrying them for sustained periods of time in the field. My talk will review these technical advances providing an overview of the potential of these new technologies to address fundamental phycology questions. I will review the sensors now capable of mapping phytoplankton health, type, size distribution and the major physical/chemical properties that regulate algal ecology. The robotic platforms that carry these sensors can now remain at sea for years capable of sampling the scale of an ocean basin. The systems are capable of mapping with the resolution centimeters and temporal resolution of seconds. Increasingly these systems are being flown as fleets that are networked and are operated as coherent network of swarming systems. The networks are increasingly being coupled to environmental forecast models allowing the scientists to proactively design sampling strategies for future conditions. I will illustrate the potential showing examples of phytoplankton responses to hurricanes, algal ecology in Southern ocean, and mapping long term trends in changing ecosystems. My take home message is that it is going to be a great time to be a phycologist!

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COCCOLITHOPHORID BLOOMS AT ELEVATED LEVELS OF CARBON DIOXIDE: NEW INSIGHTS FROM MESOCOSM STUDIES

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Increasing levels of atmospheric carbon dioxide (CO₂), caused by human activities such as the burning of fossil fuels, are driving climate change but also affect the oceans. Here, increasing levels of CO₂ are decreasing pH and carbonate saturation states, termed ocean acidification. Coccolithophores, marine calcifying phytoplankton, are key drivers of global carbon cycling, especially as calcium carbonate is known to act as ballast for the otherwise slowly sinking organic material, thus impacting marine export production. Calcification rates of several coccolithophores have been found to be negatively influenced by ocean acidification in various laboratory studies. Understanding how this might impact future ecosystem functioning and biogeochemical element

cycling is crucial. Here we report on a mesocosm CO₂ perturbation study in the framework of SOPRAN carried out in Bergen, Norway, at a time at which blooms of the coccolithophore *Emiliana huxleyi* occur naturally. Increasing CO₂ levels impacted phytoplankton community composition, favouring especially chlorophytes and cyanobacteria, while haptophytes, especially *Emiliana huxleyi*, were negatively affected. Changes in community composition were also directly reflected in particulate organic matter dynamics. Declining coccolithophore abundance/production and formation of ballasting calcite could have significant impacts for future carbon cycling by decreasing marine export production, with the potential to feed back to climate.

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ULTRASTRUCTURE OF CORTICAL AND EXTRACELLULAR STRUCTURES OF ZOOXANTHELLAE DURING CELL DIVISION IN A CORAL CELL

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Symbiotic dinoflagellates (zooxanthellae) of the genus *Symbiodinium* are found in various scleractinian corals. However, little is understood concerning the exact ultrastructure of zooxanthellae within host coral cells. This study showed changes in the cortical and extracellular structures of zooxanthellae when the cells divided in the coral cytoplasm of *Pocillopora damicornis*. Freeze-substituted zooxanthellae had typical cell coverings of dinoflagellates. Two distinct types of membrane-like layer, an outer layer A and an inner layer B enclosed the whole zooxanthella cell. Membrane fragments occurred between the layers A and B. Freeze-fracture electron microscopy revealed that the layer A consisted of biomembrane while the layer B was not of a membrane. Cytokinesis of a zooxanthella cell occurred by plasma membrane furrowing. This was followed by the constriction of the layer B between the two daughter cells. After the layer B was divided into two parts, then the layer A was constricted between the two parts of the layer B. As a result, two zooxanthella cells formed in the coral cell, each of which were completely covered with double layers of A and B.

A RECEPTOR-LIKE KINASE RESPONSIBLE FOR THE PROGRESS OF SEXUAL REPRODUCTION OF A CHAROPHYCEAN ALGA, *CLOSTERIUM PERACEROSUM-STRIGOSUM-LITTORALE* COMPLEX

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The *Closterium peracerosum-strigosum-littorale* complex is a group of unicellular charophycean alga, having morphologically indistinguishable two sexes: mt+ and mt-. Sexual reproduction is induced when cells of these two sexes are cultured together in nitrogen-depleted medium in the light. Two glycoproteinaceous sex pheromones (PR-IP and PR-IP Inducer) involved in the progress of sexual reproduction have been well characterized. Through the microarray analyses, we identified a gene encoding a novel receptor-like kinase and named CpRLK1. The CpRLK1 protein was produced after the mixing of both mating-type cells and was also detected only in mt+ cells in the presence of PR-IP Inducer. Confocal laser microscopy using CpRLK1-specific antibody revealed that CpRLK1 proteins were localized on the conjugation papilla of one of the paired cells. Phylogenetic analysis using the kinase domain revealed that CpRLK1 was closely related with CrRLK1L-1 subfamily, which was linked to the control of cell elongation of higher plants. Transformants expressing antisense-CpRLK1 gene showed delay in zygote formation, suggesting that intercellular communication by CpRLK1 protein would be responsible for the progress of the conjugation.

ELEMENTAL AND CHEMICAL CHARACTERIZATION OF RHODOLITH SAMPLES FROM THE NW GULF OF MEXICO AND PANAMA

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The surface, internal structures, and composition of rhodoliths and unconsolidated rubble collected in the northwestern Gulf of Mexico offshore Louisiana at depths of 45-85 m before and after the April 2010 Deepwater Horizon oil spill disaster were analyzed for the presence of microbionts, including cysts, bacteria, larvae, and a suite of single celled organisms using a Hitachi S-3000 Scanning Electron Microscope (SEM). In addition, Energy Dispersive X-ray Spectroscopy (SEM-EDS) was conducted on the surface and at various depths of each nodule resulting in an element spectrum profile. Preliminary data indicate a trend towards oxygen decrease following the DWH oil spill. Current research is underway to understand the different processes of element uptake by crustose coralline algae as a function of their role in rhodolith formation, taxonomic identity, geographical distribution, spatial and temporal dynamics and abiotic and biotic interactions.

A RE-EXAMINATION OF *PALISADA IRIDESCENS* (M.J. WYNNE & D.L. BALLANTINE) K.W. NAM (CERAMIALES, RHODOPHYTA) FROM THE CARIBBEAN SEA: MORPHOLOGICAL AND MOLECULAR EVIDENCE

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Specimens of *Palisada iridescens* (Rhodomelaceae) from the Mexican Caribbean were studied based on morphological and molecular characters. The original description of this taxon was as *Laurencia iridescens* M.J. Wynne & D. L. Ballantine (1991) from Guadeloupe, French West Indies. It was later transferred to *Palisada* by K.W. Nam (2007). The

phylogenetic position of this species was inferred by analysis of chloroplast-encoded *rbcL* gene sequences of 39 taxa, using one Ceramiacean taxon as an outgroup. The molecular phylogenetic results corroborate the taxonomy of *Laurencia sensu lato*, which comprises the genera *Chondrophyucus*, *Laurencia*, *Laurenciella*, *Palisada*, *Osmundea* and *Yuzurua*. In the present work the authors have found that populations of *Palisada iridescens* are more closely aligned with the recently recognized genus *Yuzurua*. We describe and illustrate morphological characteristics of our samples, comparing them with related species and also inferring the phylogenetic position. Observations on male plants are described for the first time for this taxon.

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EXTANT OR EXTINCT TIPPING POINTS – RANGE EDGE POPULATIONS AS EVOLUTIONARY HOTSPOTS

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Rear edge populations may represent hotspots of unique genetic biodiversity with high speciation potential, but may be living on the edge of extinction, pressed to develop novel means for persistence, adaptation or further range shifting. Here we describe case studies of marginal populations and species of fucoid and kelp seaweeds from the NE Atlantic, whose evolution has been shaped by range variations that left genetic fingerprints. Southern range-edge populations across various species generally contained private genetic variation. Diversity ranged from low, coincident with current population regression, to the most diverse populations across each species range, concomitant with long term persistent range areas. We report evidence for colonization fronts, range shifts, priority effects. These have been shaping genetic patterns that are unique and diverse with sharp genetic discontinuities that persist as signatures of the

colonization past. Such biodiversity patterns shaped by past evolutionary processes may now be approaching tipping points of no return, as some of the studied populations are now extinct.

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AFFINITIES OF THE HAWAIIAN FRESHWATER MACROALGAL FLORA

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The Hawaiian Islands represent the most isolated island archipelago in the world, more than 3,500 km from the nearest continent. Freshwater algae were collected from almost 1,800 sites across the Main Hawaiian Islands as part of the Hawaiian Freshwater Algal Biodiversity Survey (2008-2013). Sequence data were generated for one or more markers for several hundred of these collections, which were used, in combination with morphological/anatomical observations, to examine the systematics and biogeography of the Hawaiian freshwater macroalgal flora. The flora is composed of a mix of presumed endemic and cosmopolitan taxa, with a number of previously unknown algae being discovered for both the green (e.g. *Rhizoclonium* and *Spirogyra*) and red algae (*Kumanoa* gametophytes and the form genus *Chantransia*). Sexual reproduction appears to be lacking or infrequent in many Hawaiian freshwater algae, which means that microscopy-based species-level identifications are sometimes impossible. This, in combination with a lack of well-populated reference frameworks of comparative sequence data, means that many collections could only be identified to genus, although sequence data can give an indication of the amount of diversification within the archipelago.

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BIOGEOGRAPHIC PATTERNS OF CRYPTIC DIVERSITY IN THE GENUS *ACTINOTRICHIA* (GALAXAURACEAE) FROM THE WESTERN PACIFIC

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The red algal genus *Actinotrichia* Decaisne is currently comprised of four species which are distributed widely throughout tropical and sub-tropical reefs in the Pacific and Indian Oceans. Sequence analysis indicates that the genus contains a high degree of cryptic diversity. First, we explore different methods of species delimitation using sequences from throughout the distribution range of the genus. Second, we assess *Actinotrichia* species diversity for western Pacific Islands and investigate geographical and environmental parameters that explain the observed biodiversity pattern best.

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GENETIC STRUCTURE AND DIVERSITY AMONG POPULATIONS OF *SKELETONEMA MARINOI* (BACILLARIOPHYTA) IN THE BALTIC SEA

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Marine eukaryotic microalgae have traditionally been regarded to consist of panmictic populations. This view has been challenged by recent work showing that populations of several species are genetically structured between geographic locations. The level of genetic diversity has also been shown to vary. The harsh and geographically isolated ecosystem in the Baltic Sea is generally thought to maintain genetically distinct populations of low genetic diversity in comparison to the adjacent North Sea. We investigated the population structure and genetic diversity of the common spring bloom diatom, *Skeletonema marinoi*, along the Baltic salinity gradient. Our data set consists of 300 individual strains originating from ten locations. Eight polymorphic microsatellite markers revealed that *Skeletonema marinoi* consisted of one northern and another southern population. The level of genetic diversity increased from north to south with increasing salinity. These results indicate that dispersal barriers and marginal habitat conditions also shape diversity patterns of free-living marine microalgae. We discuss the implications of the generated information on phytoplankton population structure in relation to potential effects of climate change on the Baltic Sea ecosystem and biodiversity therein.

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EVOLVING OUT OF THE WATER: UNCOVERING THE HIDDEN DIVERSITY IN AEROPHYTIC GREEN ALGAE

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Terrestrial, aerophytic and symbiotic algae are characterized by their simplified morphology. Since they live in a specific environment, a great majority of those algae tend to have a simple, coccoid cell organization. Accordingly, due to lack of any relevant discriminative morphological characters there is still a high proportion of hidden, formally undescribed or even unknown diversity. New lineages are being identified and described as higher taxa, including some molecularly yet uncharacterized organisms that have been known for over a century. For example, the new lineage Scotinosphaerales (Ulvoephyceae) has been described recently to accommodate a distinct and highly divergent clade of *Scotinosphaera* species. Within the Trebouxiophyceae, extended sampling and molecular characterization of *Chlorella*-like microalgae isolated from the tropical and subtropical subaerial habitats led to the discovery of several independent lineages, described as new genera (e.g., *Kalinella*, *Leptochlorella*, *Xylochloris*). In addition, a substantial amount of hidden diversity has been discovered within well-defined genera of aerophytic and lichen-forming algae. In both *Asterochloris* (Trebouxiophyceae) and *Klebsormidium* (Klebsormidiophyceae), more than 70% of recovered lineages cannot be assigned to any previously described species. In addition, the vast majority of morphological features traditionally considered as taxonomically important fail to differentiate among the lineages. However, our results strongly indicate that ecological traits are reliable and phylogenetically significant in both genera, enabling the ecological circumscription of cryptic species. The ecological differentiation of related species often does not correlate with the CBC (Compensatory Base Change) species concept, which seems to be unsatisfactory for

general application. In this contribution we summarize recent achievements in uncovering the hidden diversity at both the species and higher taxonomic level.

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TRACING THE EVOLUTION AND SPECIES DIVERSITY WITHIN THE GENUS *SYNURA* (CHRYSOPHYCEAE)

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The genus *Synura* (Synurales, Chrysophyceae) represents a freshwater colonial flagellate covered with an armor of imbricated silica scales. The taxonomy of the genus *Synura* is traditionally based on morphology of the silica-scaled structures. To improve our knowledge about the evolution and genetic relationships of *Synura* species, we conducted the updated phylogeny of the genus, based on the SSU rDNA and rbcL data. The results indicate both the artificial infrageneric classification and the incorrect species concept underestimating the real biological diversity. The presence of cryptic diversity was revealed within several nominal *Synura* species, in particular in *S. petersenii* s.l. To investigate the hidden diversity in detail, we conducted a separate analysis of ITS rDNA data, including the sequences obtained from newly isolated strains sampled in lakes and ponds in Greenland, and in Northern and Central Europe. In total, four new clades within *S. petersenii* s.l. were recognized, representing novel, yet undescribed cryptic species. Transmission electron microscopy (TEM) of silica scales was used to morphologically distinguish all four clades. In addition, we detected an unexpected, extremely restricted distribution of a single clade having long and narrow scales resembling those of *S. australiensis*. Despite intensive investigation in Europe, including a close territory of Scotland, this clade occurs strictly in Ireland.

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WITHIN SPECIES CHEMICAL VARIABILITY IS IMPORTANT TO PHARMACOLOGICAL STUDIES IN *LAURENCIA* COMPLEX

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The red algae belonging to the *Laurencia* complex (Ceramiales, Rhodophyta) are known as prolific sources of halogenated secondary metabolites, particularly terpenes and acetogenins, many with important pharmacological potential. In order to correlate chemical diversity and pharmacological potential, this work evaluated the chemical profiles of crude extracts from different populations of four species within *Laurencia* complex along Southeast Brazilian coast and tested their antileishmanial activity. Our results demonstrated the presence of four major groups of chemical profiles according to the presence of leishmanicidal terpenes chamigranes (-)-elatosol and obtusol. The chemical variation observed within species support the existence of different chemotypes. However, they display a variable antileishmanial activity which was not correlated to the presence of the major compounds (-)-elatosol and obtusol, suggesting that other compounds are involved in the pharmacological action. This work showed that chemical variation within species should be taken in account in pharmacological studies involving tests with crude extracts.

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UNVEILING *LOBOPHORA* (DICTYOTALES, PHAEOPHYCEAE) SPECIES DIVERSITY IN AUSTRALIA BASED ON RBCL AND COX1 DNA SEQUENCE ANALYSES

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The genus *Lobophora* is one of the most common and geographically widespread brown algal taxa in the world. As such *Lobophora* can be found across the majority of tropical to temperate benthic marine communities. However, worldwide, there are only nine recognized species in the genus, among which *L. variegata* is unique for being known as one of the most

widely distributed marine macroalgal species in the planet. For many years this was the only *Lobophora* species known for the entire Australian flora. However, a couple of recent morphology based studies and one molecular-assisted work reported the presence of 3 other *Lobophora* species in Australia: the resurrection of *L. nigrescens* and the description of a two new species, *L. rickeri* and *L. australis*. Consequently, 4 *Lobophora* species are currently accepted in Australia. In this study, we further explored the diversity of *Lobophora* species diversity in Australia based on rbcL and cox1 DNA sequence analyses from samples collected widely across the country. GMYC (Generalized Mixed Yule Coalescent) analyses of 312 *Lobophora* rbcL DNA sequences revealed the existence of 35 (31-44) species based on single threshold model and 45 (31-45) species based on the multiple threshold model, while the ABGD (Automatic Barcode Gap Discovery) method suggested 29 species with the prior maximal distance between 1.6-3.5%. The cox1 dataset (= 52 DNA sequences) showed high congruence with the rbcL results. We combined results from both markers and propose the recognition of 33 *Lobophora* species (as Primary Species Hypotheses) for the Australian flora. Whether these species can be differentiated morphologically or not requires further studies. Nevertheless the total number of genetically distinct species of *Lobophora* in Australia, and the world, seems to have been highly underestimated. An international effort to collect and unveil the true species diversity in this genus is called for.

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COEVOLUTIONARY ARMS RACES BETWEEN SEAWEED CHEMICAL DEFENSES AND HERBIVORE OFFENSES

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Seaweeds commonly produce chemical defenses that minimize the impact of consumers, who must evolve physiological mechanisms to tolerate these metabolites. I will summarize published and unpublished evidence to argue that coevolutionary arms races between seaweeds and herbivores are rampant in marine ecosystems, and that a latitudinal difference in the quality of this diffuse coevolution has

emerged. Specifically, water-soluble versus lipophilic metabolites mediate the evolutionary interaction in temperate versus tropical systems, respectively. Recent findings also reinforce the important roles that phylogenetic lineage and geographic origin have in the feeding habits of highly generalist herbivores such as sea urchins.

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BIODIVERSITY OF MESOPHOTIC MACROALGAE IN THE PAPAHAUUMOKUAKEA MARINE NATIONAL MONUMENT, NORTHWESTERN HAWAIIAN ISLANDS

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Large macroalgal beds of the green alga *Microdictyon* were observed at mesophotic depths in the Papahānaumokuākea Marine National Monument, and appeared to form significant habitat for coral reef fish. Interest in the ecological importance of these deep macroalgal beds lead to the basic need to describe their species composition. Our objective was to describe the diversity of mesophotic macroalgae in this habitat based on collections from 2010 and 2012 cruises to the Northwestern Hawaiian Islands (NWHI). Eight locations were sampled across the archipelago from depths of 35 to 67 m by technical divers, yielding 131 collections containing 55 macroalgal species. About 30% were new species or new records. Sequence data for red algae were generated using the mitochondrial COI barcode, plastid UPA, and nuclear LSU and SSU markers, allowing phylogenetic patterns to be investigated for new species (e.g. *Dasya* and *Martensia*). Although the mesophotic flora appears to be dominated by *Microdictyon* beds, it is composed of a diverse community of red, green, and brown macroalgae, many of which are unique to the NWHI.

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INDIRECT EFFECT ON ECOSYSTEM FUNCTIONING: DECREASED PHOSPHORUS INCORPORATION NOT IRON TOXICITY DECREASES PHOTOSYNTHESIS AND GROWTH IN A GREEN MICROALGA

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The phytoplankton *Chlamydomonas acidophila* is an important primary producer in very acidic (pH 2.0–3.5), iron rich (up to 1000 mg total Fe L⁻¹) lakes. We expected the high iron concentrations to result in high iron accumulation and to inhibit photosynthesis and growth of *C. acidophila*. Therefore we grew the alga in sterilized lake water and in medium with varying iron and inorganic phosphorus (Pi) concentrations. Photosynthesis and growth of *C. acidophila* were largely unaffected by high total iron concentrations and only decreased if free ionic Fe³⁺ concentrations exceeded 100 mg Fe L⁻¹. In contrast we found an indirect effect: a concentration of 260 mg total Fe L⁻¹ (this being 15 mg free ionic Fe³⁺ L⁻¹), which is common in many acidic lakes, reduced Pi-incorporation by 50%. The resulting Pi-limitation was shown in enhanced maximum Pi-uptake rates when *C. acidophila* was grown at high iron and high Pi concentrations. Although *C. acidophila* was relatively rich in iron compared to other algae (up to 134 mmol Fe: mol C), iron toxicity was not observed. Instead, high iron concentrations result in a Pi-limitation that explains the low primary production present in iron-rich lakes.

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DYNAMICS OF PHYTOPLANKTON AND ZOOPLANKTON COMMUNITY STRUCTURES DURING SPRING CYANOBACTERIAL BLOOMS IN A SHALLOW, SUBTROPICAL LAKE IN A CHANGING WORLD

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Intensive field observations were carried out bi-weekly in subtropical, shallow, low-gradient and eutrophic Lake George, Florida, USA, during spring blooms in March – June 2009. Successions of zooplankton from rotifers to cladocerans and copepods were associated with dynamics in phytoplankton community structure that was changed from diatom-based to cyanobacteria-dominated. Changes in palatability of preferred

phytoplankton as food items to zooplankton, elevated cyanobacterial toxins (saxitoxin, microcystins and cylindrospermopsin), increased temperature, and decreased dissolved oxygen were observed and hypothesized to be factors controlling changes in zooplankton community structures. Nutrient dynamics (silica and nitrogen limitation and increased phosphorus loads) and hydrological factors associated with meteorological features in spring (increased precipitation, changes in residence time, and elevated water color) could play major roles in controlling phytoplankton community structures and indirectly affect zooplankton dynamics. These relationships between planktonic communities and environmental factors could provide insights into how anticipated climate changes (warming temperature, sea level rise, and changes in rainfall pattern) may impact the structure of planktonic communities.

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DIVERSITY OF STREAM BENTHIC ALGAE IN CALIFORNIA

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Stream benthic algae were studied from over 1,800 samples collected across California for two projects funded by the State Water Resources Control Board: (1) development of multimetric tools for setting numeric nutrient targets including an algal index of biotic integrity (2007 and 2008), and (2) surface water ambient monitoring program for the state of California (2008 to 2012). We identified 800 soft-bodied algal taxa, of which 558 to species, variety or form as follows: Cyanobacteria (273), Chlorophyta (261), Charophyta (136), Ochrophyta (52), Euglenophyta (42), Rhodophyta (20), Cryptophyta (12), and Dinophyta (4). An online identification tool has been developed for 251 species from southern California, and it is currently being updated for the entire state. Indicator species analysis based on 650 samples has identified 81 indicator species for TP, TN, DOC, copper, or reference conditions. *Spirogyra* and *Zygnema* (Charophyta) phylogeny was studied and four new to science species were described. Potentially new cyanobacterial species of *Dichothrix*, *Anabaena*, and *Bacularia* were also identified. Several rare

ochrophyte algae were recorded for the first time: *Pleurocladia lacustris*, *Chadefaudiothrix gallica*, *Chlorosaccus fluidus*, *Tetrasporopsis fuscescens*.

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HYPOXIA CAUSED BY TOXIC MICROALGAL BLOOMS

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The best known, and largest, hypoxic marine areas result from seasonal algal blooms associated with eutrophication. Less documented causes are toxic microalgal blooms and resultant benthic defaunation due to direct mortality and/or oxygen depletion. These events happen in estuaries and along coasts like the west Florida shelf. On the WFS, if there is a pycnocline present during the HAB event it can lead to hypoxia/anoxia. Two cases on the WFS will be used to characterize hypoxia events and the resultant defaunation. One, during the 1971 red tide, decimated several patch reefs off Sarasota. The second, off Tampa Bay in 2005, affected ca. 5,600 km² of bottom habitat. Data preceding, during and post events characterize the conditions leading up to the benthic defaunation and details recovery of the faunal populations. The 2005 event also demonstrates how hurricanes can influence the distribution of HABs and mixing of the water column. Both events resulted in extensive defaunation but one recovered within 2 years. These and other perturbations structure biotic communities on the WFS and affect community resiliency.

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SYSTEMATIC REVISION OF THE FAMILY OOCYSTACEAE

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The family Oocystaceae is a group of morphologically and ultrastructurally distinct green algae that constitute a well-supported clade in the class Trebouxiophyceae. Despite the clear delimitation, based on a presence of specific cell wall, only few of its members were examined using data other than morphological. After

the establishment of molecular phylogeny, the generic classification of the family was called into question. The genus *Oocystis* proved to be paraphyletic, some of the taxa were excluded and few other species newly redefined as members of this family. We investigated 46 strains assigned to the Oocystaceae using morphological, ultrastructural and molecular data (the SSU and the RbcL genes). *Oonephrys obesa* and *Nephrocytium agardhianum* cluster within the Chlorophyceae and thus are no longer members of the Oocystaceae. Conversely, we transferred to the Oocystaceae *Willea wilhelmii* from the Scenedesmaceae. On a basis of both molecular and morphological data we propose further taxonomical revisions in the genera *Franceia*, *Lagerheimia*, *Oocystidium*, *Oocystis*, *Ooplanctella* and *Neglectella*.

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INTERACTIVE EFFECTS OF SOLAR UVR AND TEMPERATURE ON THREE DIFFERENT MORPHO-FUNCTIONAL MACROALGAE GROWN UNDER MODIFIED CO₂ AND NUTRIENT REGIMES

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As part of the 2012 Aquatic Productivity (GAP) workshop, predicted climate change impacts were assessed in three species of macroalgae, *Corallina officinalis* (Rhodophyta), *Ulva rigida* (Chlorophyta) and *Cystoseira tamariscifolia* (Ochrophyta), preconditioned to high CO₂ and nutrient regimes. Experiments were conducted in outdoor tanks at combinations of carbon and nutrient enrichments representing realistic ocean acidification and eutrophication scenarios. Effects of natural (30% reduced) UV radiation were monitored before and after exposure to elevated temperatures. Short-term changes in physiological (O₂-evolution, PAM chlorophyll fluorescence) and biochemical variables (e.g., C:N, antioxidant capacity, lipids, and photosynthetic pigments) were examined. Strong diurnal fluctuations in fluorescence parameters and pigments were observed in response to ambient diurnal variation under solar radiation, and overall sensitivity

varied strongly between algal species and also depended on preconditioning treatment (carbon and nitrogen regime) in some cases. Similarly, O₂-evolution and biochemical composition exhibited significant species- and treatment-specific variation. Observed responses were linked to original habitat, algal group and morphology, and highlight the complexity of physiological and biochemical adjustments (acclimations) which occurred at different function levels and temporal scales in the three species investigated.

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TEMPORAL VARIATION OF CORTICOLOUS MICROALGAE ON A MICROSCALE

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The present study concentrated on temporal variation of the community structure and diversity of subaerial microalgae growing on bark of European Beech (*Fagus sylvatica*) in the temperate deciduous forest in the Czech Republic. The samples were taken four times: October 2010, April 2011, October 2011 and April 2012. Algal samples were cultured on agar plates and then identified to tentative OTU's by light microscopy. Altogether 44 morphotypes were recognized, mainly belonging to Trebouxiophyceae and Chlorophyceae. The largest proportion of variation in species composition of samples was explained by the temporal factors. Surprisingly, the subaerial corticolous assemblages considerably varied in time. The differences between seasons were reflected also by the α -diversity of samples. Species richness of the spring samples was considerably higher than in the autumn samples. Consequently, we concluded that the seasonal changes, along with the variation in temperature, humidity and irradiance, play a key role for the community structure of corticolous algal communities in temperate regions.

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A SURVEY OF THE BENTHIC CYANOBACTERIA OF NORTHEAST FLORIDA, USA

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Cyanobacteria are photosynthetic prokaryotes ubiquitous in aquatic ecosystems. As such, cyanobacteria are increasingly being employed as a means of assessing the health of aquatic systems. Individual taxa respond differently to environmental parameters, such as temperature, dissolved oxygen, nutrient levels, and light, and therefore, the natural fluctuation of these parameters can create a continuum of community compositions. Understanding how the cyanobacterial community responds to environmental changes, especially anthropogenic influences can be a powerful tool in management of recreational and residential waters. One of the chief impediments to employing cyanobacteria as a surrogate for ecosystem health is the lack of a baseline assessment of the natural community. While phytoplanktic communities have been sampled in the past, the benthic cyanobacterial community has been all but ignored. In order to ameliorate this dearth of data, cyanobacteria were collected from various benthic habitats in Northern Florida, such as retention ponds, rivers, springs, lakes, and streams. Thus far, ca. 100 different taxa have been found in the retention ponds alone. Of these, 29% were Chroococcales, 13% Nostocales, and 58% were Oscillatoriales. The biodiversity of the retention ponds is surprising, considering the large number of species that are seemingly fulfilling very similar niches.

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A NEW SPECIES OF *CHONDROPHYCUS* (RHODOPHYTA) FROM JAPAN

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A new species of the red algal genus *Chondrophycus* was found at Nakanoshima (36°10'N, 133°07'E), Oki Islands, Shimane Prefecture, Japan. This species does not have secondary pit-connections between epidermal cells and *corps en cerise*. The main branch appears flat or somewhat curved. Terminal branches were fork-shape or palmate. The height of mature tetrasporophytes, and female and male gametophyte

was 3.31 ± 0.1 cm, 2.20 ± 0.5 cm and 2.05 ± 0.1 cm respectively. Transparent superficial cortical cells have a few plastids, and are remarkably long in the direction of growth. Superficial cortical cells of the main branches of tetrasporophytes were 342.9 ± 96.3 μm long and 85.9 ± 16.5 μm wide. The cortical cells have a characteristic radial crystal structure substance. Tetrasporangia 167.5 ± 29.9 μm in diameter were found in main and terminal branches. Conical-shaped cystocarps 1069.3 ± 186.0 μm in diameter were present on main branches. The cup-shaped antheridial receptacles 1456.8 ± 233.4 μm in diameter were found at the tip of terminal branches. Sequences of the partial RuBisCo Large Subunit and mtDNA CoI were analyzed, and this species belongs to the genus *Chondrophyucus*.

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RE-INVESTIGATION OF *PYRAMIMONAS OLIVACEA* N. CARTER

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In 1937, Nellie Carter published “New or interesting algae from brackish water” (Arch. Protistenk. 90: 1-68.). In this paper, she described many important genera and species of phytoflagellates. Among them, *Pyramimonas olivacea* was described along with *P. angulata*, *P. obovata* and *P. octociliata* collected from a brackish pool at Bembridge, Isle of Wight, off the south of England. Since then, McFadden et al. (1987) reported electron microscopy of *P. olivacea* isolated from some localities of Australian coasts. They proposed a new subgenus based on the species, subgenus *Punctatae*. The original description of Carter (1937) clearly indicated double eyespots, however, material of McFadden et al. (1987) had a single eyespot. Hence, McFadden et al. (1987) emended *P. olivacea* from double eyespots to single eyespots. In recent survey from Japanese and South African coasts, we isolated morphologically identical strains of N. Carter’s *P. olivacea*. Hence, species of McFadden et al. (1987) may not be proper *P. olivacea*. In this presentation, we propose new emendation of *P. olivacea* N. Carter and should be treated as a new species of McFadden et al.

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LIVING COCCOLITHOPHORES IN CHINA SEA WATERS: THE DIVERSITY AND DISTRIBUTION

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We investigated the morphology, taxonomy, diversity and distribution of living coccolithophores (LCs) in the China Sea Waters (CSW) for the first time. 97 species were recorded, belonging to 4 orders, 11 families, and 44 genera. Genus *Syracosphaera* (20 species observed) presented the highest species-richness in the CSW. The overall LCs abundance in the research areas ranged from 3 to 20 cells /mL. The dominant LCs species were *Gephyrocapsa oceanica*, *Emiliania huxleyi*, *Helicosphaera carteri*, and *Algirosphaera robusta*, in the order of species abundance. The total LCs abundance was highest in the East China Sea, followed by the South China Sea, Yellow Sea, Northern Yellow Sea and Bohai Sea. Seasonally, the LCs were most abundant in autumn, followed by spring, winter and summer. Temperature and nitrate concentration may be the major environmental factors controlling the distribution and species composition of LCs in the studying areas based on canonical correspondence analysis (CCA).

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A NEW SPECIES OF *LOBOPHORA* (DICTYOTALES, PHAEOPHYCEAE) FROM THE SOUTH CHINA SEA

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Lobophora J. Agardh, a brown algal genus occurring in tropical and subtropical seas, is characterized by a marginal row of meristematic cells and a single-layered large central medulla, lacking paraphyses around sporangium sori. Our recent research suggested that the interspecific divergence is highly underestimated, and many species should be included in *Lobophora*. Herein, we report a new species

collected from Hainan Island in the South China Sea. The species grows erectly in the subtidal zone, attaching to the substrate by a basal holdfast. The fan-shaped thallus is 5-8 cm long, and 140-165 μm thick at the middle part. Thallus is commonly composed of a single layer of large medullary cells and four layers of cortical cells on both sides of medulla. Sporangium sori are primarily scattered on the ventral surface and not surrounded by paraphyses. Developed sporangia are sessile and ovate, 60-75 μm in diameter at surface view. The predominant erect thallus, four-layered cortical cells, and molecular evidence (rbcL and cox3) all showed that *the new species* is distinguished from the congeneric species.

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DO SHALLOW-WATER SEDIMENTS WITH MICROPHYTOBENTHOS CARE ABOUT WARMING?

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Sediments were exposed to warming (+4°) alone and in combination with a toxicant, nutrient enrichment, or acidification. Sediment-water fluxes of oxygen, alkalinity and nutrients (light/dark), primary and bacterial production, denitrification, N mineralization, biomass and composition of organisms were measured. Autotrophic variables were less affected than heterotrophic variables by warming. Generally, retained net autotrophy due to microphytobenthos moderated temperature effects. The mode of interaction between warming and a second factor was non-additive (synergistic or antagonistic) rather than additive, but often no significant interactions were found. In autumn, warming alone and together with nutrients caused an earlier, but temporary, shift to net heterotrophy. The warming-toxicant interaction was antagonistic, warming removing toxicant effects on functions in light. Since benthic and floating microalgal mats responded differently, warming-toxicant effects may be habitat dependent. The results emphasize the need to consider both structural and functional variables, and both direct and indirect effects when assessing stressor interactions. Structural Equation Modelling can separate indirect and direct effects, as shown in the presentation by Christian

Alsterberg on warming-acidification effects on benthic primary producers.

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BIOSYNTHESIS AND ACCUMULATION OF HYDROCARBONS AND POLYSACCHARIDES IN A COLONIAL GREEN ALGA, *BOTRYOCOCCUS BRAUNII* RACE B

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The colonial *Botryococcus braunii* accumulates large quantities of hydrocarbons that are mainly deposited in the extracellular space, while all other examined oleaginous microalgae store lipids in the cytoplasm. We studied *B. braunii* by electron microscopy, and report 1) the behavior of lipid bodies (LBs) and the stage of hydrocarbon secretion during the cell cycle, and 2) the formation and characterization of an extracellular matrix. New lipid accumulation on the cell surface occurred after cell division at the basolateral region. LBs were increased in number and size until the new lipid accumulation would start. To form a colony, cells were connected with hydrocarbon layers accumulated at basolateral region. The outside of colony was surrounded with a 3-4 nm fibrillar colony sheath. Each fibril was stained with silver-hexamine. We examined how the colony sheath was built. After cell division, the fibrillar materials accumulated between daughter- and mother-cell wall at the cell apex concomitant with lipid accumulation at the basolateral region. After rupture of the mother-cell wall, fibril structures extended from daughter cell wall to be a part of the colony sheath.

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DISTRIBUTION AND SEASONALITY OF MACROALGAE IN MACAO

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This paper presents the results of a preliminary macroalgal baseline study in Macao, a small coastal city situated on the southern coast of China, west of the heavily industrialized Pearl River Delta region. Significant growth of the red macroalgae *Prionitis* sp. and *Gelidium* sp. were observed during the winter and spring months of November to April 2012, although geographically limited along the wave exposed rocky shores of Coloane Island. Reproductive structures (cystocarps) in *Prionitis* sp. were also observed in these months. *Ulva* sp. and other green macroalgae, significantly grow in the same winter/spring months but are more widely distributed, occurring in most of Macao's coastline. Seasonal patterns on the occurrence/growth of these species are likely to be caused by changes in temperature and salinity over the seasons while the limited geographical distribution of *Prionitis* sp. are likely to be caused by the type of substrate and strength of wave action and water movement.

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**FRAGILARIA ATOMUS
(BACILLARIOPHYTA): MORPHOLOGY
AND COMPARISON WITH RELATED
TAXA**

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Fragilaria atomus was first described by Hustedt from Finland in 1931; it has since been found worldwide. However, due to its small size it can easily be mistaken with other fragilarioid diatoms. To elucidate the morphological, metric and structural features of the species, specimens from salt marshes in Brazil and type material from the Hustedt's Collection were studied using LM and SEM. Brazilian material was obtained by scraping the stems of macrophytes, while type material (lectotype, E2963) was sampled from sediment in the Southern coast of Finland. The results revealed that diacritic features of *Fragilaria* are not present in *F. atomus* such as, rounded closing plates, rimoportula and spines. The absence of these structures, together with the opposite striation pattern,

round areolae and a reduced or absent sternum allows the transfer of *F. atomus* to the genus *Stauroforma*. The species was compared with seven morphologically similar taxa belonging to *Fragilaria*, *Stauroforma* and *Psammoneis*. The main differences between *S. atomus* and these taxa are related with the number of striae, the kind of striation (opposite or alternate) and the shape of axial area.

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THE DILEMMA OF GYMNODINIUM

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The genus *Gymnodinium* was established in 1883 by Stein based on the type species *Gymnodinium fuscum* (Ehrenberg) Stein 1878 and emended by G. Hansen and Moestrup in 2000. This genus currently contains 234 extant, described species. There are now several major issues associated with this genus including that it has both freshwater and marine species; it is polyphyletic, the emended description leaves many previously described species now uncertain; life history stages may have been described as species; and some described species may not be *Gymnodinium*. It is estimated that 10%-25% of the species currently assigned to *Gymnodinium* are not valid. Molecular techniques are currently unable to clarify the taxonomy because only 7% of *Gymnodinium* species have a sequence in GenBank. The problem is exacerbated by the lack of proper naming of sequences that prevents proper assignment of all available data. Only 30% of the *Gymnodinium* sequences in GenBank are labeled with a proper scientific name. These findings have implications for microbial eukaryote biodiversity estimates and agree with additional findings that protist diversity may be overestimated. In this poster we describe the current state of the genus, discuss the issues and propose next steps.

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**EFFECTS OF IRRADIANCE AND
TEMPERATURE ON GROWTH,
PHOTOSYNTHESIS AND TOXIN
PRODUCTION OF TOXIC DIATOM
PSEUDO-NITZSCHIA AUSTRALIS
(BACILLARIOPHYCEAE)**

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The marine diatom *Pseudo-nitzschia australis* which produces domoic acid (DA), a toxin causing Amnesic Shellfish Poisoning in humans, is responsible for toxic blooms in the Bay of Seine (English Channel - France). In order to better understand the influence of abiotic factors on the cell physiology of this species, the effects of irradiance and temperature on growth, photosynthetic parameters and DA production were investigated in culture. A *P. australis* strain isolated from the Bay of Seine was maintained in exponential growth phase in semi-continuous cultures under different temperature and light conditions. *P. australis* was able to grow between 3 and 21°C with an optimal growth temperature estimated at 17°C. The stability of *P. australis* photosynthetic parameters under the whole temperature gradient highlights the high thermal-acclimation capacity of the photosynthetic apparatus of this species. However, the decrease in growth rate at the highest temperatures under high light levels revealed the influence of light-temperature interactions on *P. australis* physiology. The results of our experiments also showed that *P. australis* produces DA during exponential growth phase and that DA production is promoted by high light intensities. A significant exponential relationship was observed between DA production and growth rate for higher light levels. These results should allow a better understanding of *in situ* *P. australis* growth capacities, and DA production. This work will be used to define parameters of a *P. australis* module in an ecosystem model.

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ECOLOGICAL DYNAMICS OF *ULVA* MACROALGAL BLOOMS

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Macroalgal blooms are frequent occurrences in estuarine systems worldwide. Blooms in Narragansett

Bay, RI, USA, have been reported for over a hundred years, but have increased in duration and frequency over the past few decades. At the same time, anthropogenic inputs of nitrogen into this system have been quite large (~50-60 million moles N/year), although they have decreased by over 35% due to recent updates in sewage treatment plants. We have assessed bloom formation, duration, and composition in this system for over seven years through a combination of repeated intertidal and subtidal quantitative surveys, and combined these with studies of *Ulva* overwintering strategies, growth rates and competitive interactions. While we find significant spatial and temporal (short-and long-term) variability in bloom composition and abundance (with maximum densities over 400 g/m² wet mass), our data indicate that *Ulva compressa* and *Ulva rigida* exhibit predictable temporal shifts in abundance. In addition, our laboratory and field experiments indicate that these species likely use multiple methods for surviving harsh winter conditions, including burial in sediment, suspension in the water column, and overwintering of attached microscopic stages in this system.

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EFFECTS OF SURFACE COMPLEXITY AND CONSEQUENT WATER FLOW VARIATION ON THE ESTABLISHMENT OF BENTHIC STREAM MACROALGAE

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Habitat complexity has been presented as being an important influence on different ecological communities, but its operation under variable natural conditions is not well understood, particularly in lotic habitats. To investigate the role of habitat complexity, in particular the fractal structure of surface irregularity in a stream system, field experiments were conducted using surfaces with different levels of fractal dimension in four unshaded streams in southeastern Brazil. The samplers remained completely submerged for 60 days after installation. The establishment of macroalgae was measured only at the end of the experimental period in terms of percent cover (abundance) from the area occupied by the species

associated with the substrate. The percent cover of macroalgae was statistically different among treatments. The surfaces with higher complexity recorded higher values of macroalgal abundance, and the hydraulic characteristics linked with variation in surface complexity showed an important influence on benthic stream macroalgae establishment.

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MORPHOLOGICAL PLASTICITY IN THE EPIZOIC DIATOM *FALCULA HYALINA* TAKANO FROM A COASTAL LAGOON, SOUTHERN BRAZIL

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Falcula hyalina Takano is a species which taxonomic position still remains uncertain. The species was described by Takano in 1983, using TEM. Seven years later, Round, Crawford & Mann, in the diacritic description of the genus, raised the question that *F. hyalina* had some features that do not confer with *Falcula*, such as, wider sternum and poroidal apical pore field. Afterwards Prasad et al. (1989) improved the knowledge of *F. hyalina* without drawing conclusions about its generic identity due to the impossibility of having the type of the genus for study. The studies of this species demonstrated that its valve morphology and biometrical data are similar in the different regions where it has reported. However, *F. hyalina* showed morphological plasticity in the population found in a coastal lagoon (31°26'S - 51°10'W and 31°14'S - 50°54'W). The study was based on plankton samples collected in the winter of 2009. We describe and illustrate the new morphotype in LM, SEM and TEM and inform the ecological conditions in which *F. hyalina* was observed. This record corresponds to the most austral report of the species in South America.

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LONG-TERM ACCLIMATION OF THE ANTARCTIC SEA ICE DIATOM *NITZSCHIA LECOINTEI* TO HIGH PCO₂

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Cold-water oceans have a high capacity of absorbing atmospheric CO₂. Consequently, high-latitude oceans are particularly susceptible to changes in atmospheric pCO₂, and are projected to be the first oceans impacted by ocean acidification. Sea ice diatoms play an important role in carbon biogeochemistry and polar food webs. The response of diatoms to elevated pCO₂ is mainly addressed through short-term perturbation experiments. However, little is known about the acclimation and adaptation potential of microorganisms to ocean acidification. We have performed a 200-day CO₂-perturbation experiment (280, 390 and 960 ppm CO₂) on the sea ice diatom *Nitzschia lecointei* to investigate ecophysiological acclimation to high pCO₂. We have previously reported short-term synergistic effects of increased temperature and pCO₂ on growth rate and fatty acid composition in *N. lecointei*. However, preliminary long-term data reveal different patterns in terms of primary production, e.g. suggesting increased DOC production due to carbon overconsumption at high pCO₂. Many research gaps still exist in acclimation and adaptation to global change – a key point in understanding how algal populations and carbon biogeochemistry may alter in a high CO₂ world.

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PHOTOSYNTHETIC EUGLENOIDS ON THE EUGLENOZOAN TREE OF LIFE

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The Euglenozoan Tree of Life Project focuses on relationships within and among the Kinetoplastida, Diplonemida and Euglenida (Euglenophyta). Of these divergent but related groups, only the Euglenophyta are generally considered among the algae. Photosynthetic euglenoids are believed to have acquired chloroplasts through secondary endosymbiosis with an ancestral green alga most closely related to the present day *Pyramimonas*. We have sequenced over a dozen chloroplast genomes from taxa representing all of the major photosynthetic genera to trace the evolution of the chloroplast genome within the euglenoid lineage. Phylogenomic analyses suggest that the acquisition of the chloroplast was a single event which resulted in significant gene loss and rearrangement. While gene content is highly conserved

among photosynthetic euglenoids, chloroplast genome size changes dramatically across the lineage, largely due to an increase in the number of introns, ranging from less than ten introns in *Eutreptiella gymnastica* to over 150 in some *Euglena* species. Analyses of multiple genome alignments show large scale evolutionary events with many blocks of genes undergoing rearrangements and inversions among the taxa and even within genera.

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NITZSCHIA: A COMPLEX VARIATION PATTERN HAS A COMPLEX EXPLANATION

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Nitzschia is a large genus, with several species being important from (paleo-) ecological point of view: they occur frequently and abundantly in all kinds of waters, and several of them are considered to be indicators of particular environmental conditions. However, *Nitzschia* is also notorious for its taxonomic difficulty, and members of the section Lanceolatae are particularly challenging because most have small cells and delicate structure, and because they offer few diagnostic characters. Our studies (combining morphological, molecular and reproductive data) of some *Nitzschia* spp. of section Lanceolatae (e.g. *N. inconspicua*, *N. palea*) have revealed a complex variation pattern. In each, morphological variation between strains is very subtle but molecular data (LSU, *rbcL*, some *cox1*) show moderate (*N. palea*) or considerable (*N. inconspicua*) diversity. In *N. inconspicua*, 7 genotypes from 36 isolates grouped into 3 clades. Distinct salinity responses occurred among *N. inconspicua* strains though differences were genotype- but not clade- or morphologically related. Most *N. inconspicua* strains are automictic (like some other Lanceolatae e.g. *N. fonticola*) and the biological species concept is therefore inapplicable. Furthermore, *N. inconspicua* is paraphyletic with respect to other morphologically-defined *Nitzschia* species. In contrast, deepened sampling of the heterothallic *N. palea* (> 80

clones and sequences) continues to show a robust monophyletic group with poorly resolved internal structure, though containing a wide range of stria densities (a character exhibiting low intraclonal variation in several Lanceolatae). Our results suggest that, at least for the *Nitzschia* Lanceolatae group, formulating rules for molecular species recognition (and DNA barcoding) should be done cautiously, informed by an understanding of phylogeny, functional significance and reproductive biology.

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INFERRING THE PHYLOGENETIC POSITION OF PALMOPHYLLALES USING THE GENOME OF *VERDIGELLAS PELTATA*

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Recent molecular studies provided evidence that the genera *Verdigellas* and *Palmophyllum* (Palmophyllales, Chlorophyta) form a distinct and early diverging lineage of green algae. Although phylogenetic analyses supported a deep-branching Palmophyllales, its exact phylogenetic placement is still uncertain. In order to elucidate the phylogenetic position of this group of deep-water algae, we conducted a multigene partitioned Bayesian analysis, including chloroplast single-copy genes (*petA*, *petD*, *ycf3*) and *rbcL*, as well as 18S rDNA, with a broad sampling of taxa. Chloroplast and nuclear genes were extracted from a draft genome assembly of a sample of *Verdigellas peltata* dredged offshore NW Gulf of Mexico. Phylogenetic analyses placed *Verdigellas* as basal to both Streptophyta and Chlorophyta, indicating that Palmophyllales might be the earliest branching lineage of the Viridiplantae.

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CHARACTERIZATION OF CONJUGATION PROCESSES IN HOMOTHALLIC *CLOSTERIUM PERACEROSUM-STRIGOSUM-LITTORALE* COMPLEX

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The *Closterium peracerosum* – *strigosum* – *littorale* complex is a unicellular charophycean alga, in which there are two modes of zygospore formation: heterothallism and homothallism. In the heterothallic strains, zygospores are formed as a result of conjugation between mt+ and mt– cells. In contrast, zygospores of homothallic strain are formed within clonal cells. In this study, conjugation processes of a homothallic strain were critically analyzed. Time-lapse analyses revealed that most of the observed zygospores were originated as a result of conjugation of two sister gametangial cells derived from one vegetative cell. When the homothallic cells were mixed with phylogenetically closely related heterothallic cells (mt+ or mt–), which had been vitally stained using calcofluor white, formation of hybrid zygospores between the homothallic cells and heterothallic cells were confirmed. These results indicate that some of the homothallic gametangial cells possess heterothallic mt– and/or mt+ like characters. It could be speculated that the division of one vegetative cell into two sister gametangial cells is a segregative process that produces complementary mating types equivalent to mating types in heterothallic strains.

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ECOLOGICAL STUDY OF THRAUSTOCHYTRIDS (STRAMENOPILES, LABYRINTHULOMYCETES)

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Thraustochytrids are colorless marine heterotrophs (Labyrinthulomycetes, stramenopiles). They are beginning to attract attention as the important decomposers in the marine ecosystem, because of existence in the ocean around the world, high-speed growth and oil accumulation. However, almost all reports on the thraustochytrid biomass have been not based on the continuous monitoring. In this study, we investigated the seasonal changing of biomass and

species composition of thraustochytrids once a month for five years in three fixed points at the mouth of Shukugawa River and two points in Osaka Bay, Japan. It was observed a larger number of thraustochytrids at the river mouth than the inside of the bay. At the river mouth, the comparison of the carbon biomass between thraustochytrids and bacteria suggests that the contribution of thraustochytrids to the microbial loop cannot be ignored. Phylogenetic position of isolated strains clearly shows the repeated seasonal succession of thraustochytrid species, but species compositions of the river mouth and two other points are different. It probably suggests that each species differs in the source of nutrient.

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DISTRIBUTION, VARIATION, AND PHYLOGENY OF SAXITOXIN BIOSYNTHESIS GENES IN THE DINOFLAGELLATE GENUS GYMNODINIUM

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The dinoflagellate *Gymnodinium catenatum* is the only gymnodinoid saxitoxin-producing dinoflagellate and, as such, is phylogenetically distant from all other saxitoxin producers that belong exclusively to the related gonyaulacoid genera *Alexandrium* and *Pyrodinium*. Recent work indicates that the gene encoding the key initiating synthetic step (SxtA) of *G. catenatum* is relatively closely related to that of *Alexandrium* species, raising the hypothesis that the capacity for saxitoxin synthesis in *G. catenatum* arose via recent lateral transfer of SxtA and/or other biosynthetic genes either from cyanobacteria or other dinoflagellates. To determine to possible origin of saxitoxin biosynthesis we used PCR to screen several *Gymnodinium* species for the presence of the A1 and A4 domains of SxtA, including the related species *G. nolleri* and *G. microreticulatum* and the non-toxic gymnodinoid species *G. aurelolum* and *G. impudicum*. The sxtA1 and/or sxtA4 domains were detected only in strains of *G. catenatum*. We propose that the evolutionary origin of the saxitoxin production gene in *G. catenatum* was in an ancestral bacterium, that the

Gymnodinales and Gonyaulacoid PSP-producing dinoflagellates shared the same common ancestor of the sxt gene and that they have subsequently evolved independently in parallel manner. The introduction of STX in *G. catenatum* is evidently at the same time as the divergence of *G. catenatum* from the *G. catenatum* complex. Furthermore, the saxitoxin gene in cyanobacteria and dinoflagellate had evolved independently and they do not share the same common ancestor.

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THE CHIMERIC NATURE OF CHROMALVEOLATE PLASTIDS

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Through secondary endosymbiosis plastids of chromalveolates stem from a red algal plastid and consequently they contain a red algal plastid genome. However, compared to extant red algal plastid genomes those from chromalveolates lack about 50 genes. During the process of secondary endosymbiosis also a number of nuclear encoded plastid genes were transferred from the red algal nuclear genome to the chromalveolate nuclear genome through endosymbiotic gene transfer (EGT). Taken together, however, identifiable red algal plastid genes in chromalveolates are insufficient to run the plastid. Seemingly this was overcome by a massive introduction of non-red algal plastid genes, most of them affiliated to Prasinophytes, primitive chlorophytic algae. As a consequence extant chromalveolate plastids are no longer rhodophytic but chimera of red algal and green algal plastids and they possess novel functions not known in their ancestors, rhodoplasts.

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ACTIVATED DEFENSES AND TOXINS IN TEMPERATE BLOOM-FORMING GREEN ALGAE: WHAT YOU SEE ISN'T ALWAYS WHAT YOU GET

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Ulvoid green algae are conspicuous components of macroalgal blooms in Washington State. The natural products that they produce affect herbivore grazing and the growth and survival of organisms in the surrounding seawater. Two groups of compounds, based on dimethylsulfoniopropionate (DMSP) and dopamine, are stored in the algal cells as precursors and broken down by enzymes into more toxic or deterrent compounds. In the case of DMSP, a lyase enzyme cleaves the molecule into DMS and acrylic acid. In the case of dopamine, a phenolase enzyme catalyzes the oxidation of dopamine into quinones. We have examined the environmental conditions that cause the breakdown of the stored precursors and the release of the biologically active products. Dopamine breaks down in response to physical damage and damage from desiccation. DMSP breaks down in response to grazing, low salinities, and high temperatures. Because specific environmental conditions are required to cause the enzymatically mediated release of the toxic or deterrent products of the stored precursors, measurements of the precursors may not provide an accurate assessment of their impacts in the field.

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REDOX-BASED SENSING OF ENVIRONMENTAL STRESS – FROM ORGANELLE SIGNALING TO CELL FATE DECISION

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Diatoms are one of the most successful groups of photosynthetic protists in the modern oceans, responsible for about 20% of global primary productivity. The molecular basis for diatom's ecological success and the role of cell signaling are still poorly understood, although recent studies suggested that diatoms utilize sophisticated sensing mechanisms to respond to environmental stress conditions. During bloom succession algal cells are subjected to abiotic (nutrients deprivation, high light) and biotic stress (viruses, grazers and allelopathic interactions). Production of Reactive Oxygen Species (ROS) under stress conditions and consequently

alterations in cell redox state have been shown to play a central role in regulation of cell fate signal transduction pathways in plants and animals. We therefore explored diatom mechanisms of perception of stress conditions by combining in vivo imaging of redox response with quantification of the whole redox proteome (redoxome) in the model diatom *Phaeodactylum tricornerutum*. In vivo imaging of the redox state in various subcellular compartments, using the redox sensitive GFP (roGFP), revealed distinct compartmentalized signaling in response to light regime, nitrogen or iron availability as well as infochemicals that are derived from diatom biotic interactions. We further identified intriguing correlations between early oxidation patterns in the mitochondria and subsequent induction of cell death. Using a redox proteomics approach we were able to unravel the redox-sensitive protein network which includes key enzymes in diatom metabolic pathways. Comparative analysis of the diatom redoxome across 48 genomes revealed reactive cysteines that are evolutionarily conserved across kingdoms. We propose that redox regulation may provide diatoms with important machinery for rapid and reversible responses to multiple environmental cues therefore essential for their ecological success in the marine ecosystem.

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MARKER DEVELOPMENT AND POPULATION GENETIC STRUCTURE OF *PORPHYRA* SPP. IN THE PENINSULA IBERICA

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In order to examine the population genetic structure of *Porphyra* spp. in the Iberian Peninsula, nine polymorphic microsatellite loci were isolated from several enriched DNA libraries in *Porphyra* species from Portugal: *Porphyra umbilicalis* and *Porphyra linearis*, and they were optimized for future studies of its population genetic structure across the North Eastern Atlantic. The newly developed markers were screened for variability among more than 100 individuals from different locations mainly in Portugal

and Spain. Tests for cross-amplification in *Porphyra dioica* were also performed. For *Porphyra linearis*, allele number ranged from 3 to 15 per locus, and with estimates of total heterozygosity varying from 0.06 to 0.86. These markers will provide an opportunity to explore the genetic variability and biogeography in the *Porphyra/Pyropia* genus.

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MORPHOLOGICAL VARIATION AND MOLECULAR CONFIRMATION OF THE RED ALGAE *HYPNEA MUSCIFORMIS* (CYSTOCLONACEAE, RHODOPHYTA) FROM YUCATAN PENINSULA, MEXICO

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The genus *Hypnea* exhibits large morphological variation resulting in great confusion concerning its taxonomy. With over 71 species currently accepted taxonomically and 41 synonyms, the major challenge today is to integrate morphological and molecular information in order to assign the proper species names. *Hypnea musciformis* is widely distributed along the Atlantic, the Pacific and Indian Oceans. Taxonomical confirmation for each region is needed in order to discard the presence of cryptic species due to the morphological variations mentioned. In Yucatan peninsula *H. musciformis* has been reported, however, no morphological description or molecular confirmation is available for this region. This work reports the great morphological diversity found in *H. musciformis* from Yucatan peninsula, including ecological, culture observations and molecular analysis. RbcL (ribulose biphosphate carboxylase gen) and cox1 (cytochrome oxidase I gen) analysis confirmed the taxonomic identity of the specie showing that variations in morphology are probably due to plasticity. Examination of specimens from different regions (Gulf of Mexico, western Atlantic coast and Mediterranean coast) allowed the identification of the relevant morphological characters for the taxonomical analysis of this species.

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EVOLUTION OF THE THERMAL NICHE AND ITS IMPACT ON BIODIVERSITY PATTERNS IN SEAWEEDS

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The thermal niche of seaweed species is known to have a strong influence on their distribution ranges. In turn, geographical distributions determine patterns of species richness across the globe. But the thermal niche is not a static feature over evolutionary timescales. Our goal is to incorporate the evolutionary dynamics of thermal niches into assessments of global patterns of species richness. Using evolutionary modeling approaches in a phylogenetic context for three seaweed genera, we show that the thermal niche evolves at different rates in different lineages, and illustrate relationships between microhabitat preferences and rates of thermal niche evolution. We show that niche evolution promotes the diversification of taxa by different mechanisms and reveal its impact on global diversity patterns. In summary, our results show that the thermal niche evolves in a highly taxon-specific manner and that it has a clear impact on the species richness patterns of those taxa.

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THE ROLE OF ADAPTIVE AND NON-ADAPTIVE PROCESSES IN MARINE ALGAL SPECIATION

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Only recently, by a combination of adopting molecular techniques and global sampling, phycologists started to obtain a meaningful idea of algal species diversity, the distribution of the individual species and ultimately patterns of diversity. The emerging information enables us to test hypotheses on the modes of speciation and the role of a divergent natural selection on adaptive traits in diversification. Here we use a

global dataset containing over 3000 DNA-verified specimen records belonging to the Dictyotales to elucidate the mechanisms that drive speciation in this brown algal order. The focus is on tropical and warm temperate environments. We characterize the taxonomic structure on large geographic scales (between and within ecoregions) and examine the predictions of various geographic (allopatric, sympatric, and peripatric) models of speciation, using patterns of range overlap and range size symmetry between sister clades. Adaptive processes are addressed on a more local scale in New Caledonia. Focusing on the genus *Lobophora*, we examine up to which extent individual sympatric species are adapted to specific environments. Many species of *Lobophora* engage in allelopathic seaweed-coral interactions, with coral bleaching and mortality resulting from direct contact. We quantify coral-algal interactions by mapping host occupancy of the individual *Lobophora* species and by conducting field experiments whereby coral hosts are exposed to lipid-soluble extracts from different *Lobophora* species. Results from geographic speciation models and adaptation to specific environments at a local scale are combined to draw conclusions on the relative roles of adaptive and non-adaptive processes on diversification of seaweeds.

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ASSESSMENT OF FRESHWATER RED ALGAL (THOREALES AND BATRACHOSPERMALES, RHODOPHYTA) TYPE SPECIMENS IN THE HERBIER CRYPTOGAMIE

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The Batrachospermales and Thoreales are macroscopic algae inhabiting freshwater streams worldwide. French naturalists, such as Bory de Saint Vincent, Montagne, LePrieur and Sirodot, were among the first to collect and study these organisms. Their specimens from 18th and 19th centuries are within the sub-herbaria (Thuret, Montagne, General France, General World and Sirodot) in the Herbarium Cryptogamie (PC), Muséum d'Histoire Naturelle, Paris. Recently, researchers have utilized the collections and referred to them in publications. However, most type material was not annotated and due to duplicate specimens within and

among sub-herbaria, relocating this material is difficult. Specimens are now being high-resolution scanned, barcoded and databased making it easier to locate specimens. Among the sub-herbaria, there is type material for 32 currently recognized species/varieties many of which are represented by lectotypes, syntypes and topotypes. The Sirodot collection consists of hundreds of previously unexamined specimens, almost all of which would have some type designation. In addition, specimens of rare taxa have been located and will be designated as lectotypes. Overall, the PC collection is an important resource with ~3000 freshwater red algal specimens.

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OPTIMIZING ALGAE BIOFUELS: ARTIFICIAL SELECTION TO IMPROVE LIPID SYNTHESIS

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Algae biofuels are a promising alternative to petroleum-based fuels, but algae oil yields must improve to make implementation economically feasible. Here, artificial selection is investigated as an original approach to increase algae lipid production. To heighten the activity of acetyl-CoA carboxylase (ACCase), an enzyme crucial to lipid synthesis, artificial selection was used to isolate cell lines tolerant to the ACCase-inhibiting herbicide sethoxydim. Incremental nitrogen deficiency was implemented as a control condition to refine GC-MS lipid analysis, ACCase immunoblotting, and a spectrophotometric ACCase enzymatic assay. These techniques were applied to determine changes in lipid metabolism caused by acquired sethoxydim tolerance. Developed lipid analytical protocols show repeatability. NMR spectroscopy indicates efficient fatty acid esterification. Anti-biotin immunoblotting allows visualization of ACCase. Significant increases in both lipid accumulation and sethoxydim tolerance were observed in selected cultures even after multiple generations out of selection, indicating potential success in selecting for algal cell lines with heritably increased ACCase and lipid production. If such populations can be sustained, artificial selection could prove a novel method to increase microalgae oil yields, and thus the viability of algae biofuels.

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PHYLOGENETIC RELATIONSHIPS AMONG DIFFERENT POPULATIONS OF FRESHWATER BROWN ALGAE *HERIBAUDIELLA FLUVIATILIS* AND *BODANELLA LAUTERBORNI*

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Of an estimated 2,000 brown algae species (Phaeophyceae), less than 1% occur in freshwater habitats. Freshwater brown algae have been known for more than 150 years, but the phylogenetic relationships and taxonomic placement of some species remains unclear. Currently, there are no comprehensive molecular phylogenetic studies of freshwater brown algae, which would provide additional insight into evolution within this class. This study aims to resolve the phylogenetic positions and better understand the relationships among populations of the freshwater brown algae *Heribaudiella fluviatilis* (Areschoug) Svedelius (1930) and *Bodanella lauterborni* Zimmermann (1927). *Heribaudiella fluviatilis* has been reported in Europe, Asia, and North America, and *B. lauterborni* has been found only in Europe. Although quite different morphologically, these two species were found to be relatively similar genetically. Variable regions from the organellar genomes of these two species were selected as molecular markers to compare different individuals of *H. fluviatilis* collected from North America and from Europe, and *B. lauterborni* from Europe. Phylogenetic relationship generated using both mitochondrial and plastid markers support *H. fluviatilis* from North America as sister to *B. lauterborni* from Europe, and *H. fluviatilis* from Europe sister to these. Three possible taxonomic conclusions inferred from this result will be discussed, including: (1) although *B. lauterborni* is morphologically distinct from *H. fluviatilis*, these three lineages might represent different phenotypes of one polymorphic species (*H. fluviatilis sensu lato*); (2) these lineages might represent three distinct species within *Heribaudiella*; (3) these three lineages might each warrant separate generic status.

SUCCESSFUL INVADERS ARE BETTER DEFENDED: THE EXAMPLE OF *GRACILARIA VERMICULOPHYLLA*

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To evaluate the importance of anti-herbivore resistance for algal invasion success we compared resistance traits among specimens of the red macroalga *Gracilaria vermiculophylla* from six native populations in Korea and China and eight invasive populations in Europe and Mexico that were maintained under identical conditions in the laboratory. Herbivorous snails both from the native range (*Littorina brevicula*) and from the invaded range (*Littorina littorea*) consumed significantly less of seaweed specimens originating from non-native populations. Metabolome profiling revealed that this preference was correlated with an increased wound-activated production of deterring prostaglandins and hydroxyeicosatetraenoic acids. Thus, invasive populations of *G. vermiculophylla* are more strongly defended against challenge by herbivores and other biological enemies that cause local tissue or cell disruption and activate oxylipin production. Anthropogenic distribution of genotypes adapted to resist elevated feeding pressure probably contributed to the invasion success of this species.

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POPULATION ECOLOGY OF *PALMARIA PALMATA* ON DIGBY NECK, NOVA SCOTIA CANADA

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Population ecology of *Palmaria palmata* (dulse) was investigated in Digby Neck, Nova Scotia, where commercial harvesting occurs. The primary objectives of this study were to determine: 1) the reproductive phenology of dulse, 2) the seasonality of vegetative

development between epilithic and epiphytic populations and 3) the average growth rate. Each month for two years, fifty representative fronds were collected from each dulse beach and the reproductive state was determined. We found that reproductive maturity of the fronds peaked in the late fall with the majority of the frond being identified as male or tetrasporophytes. Fifteen of the largest thalli were collected from epiphytic and epilithic populations each month for 6 months. There was no significant difference between the fronds in different habits and the proportions of fronds in each size category were similar. All thalli had a high number of smaller fronds (between 1 cm and 5 cm) and a low number of larger fronds (25 to 30 cm or over 31 cm). The average growth rate of vegetative fronds in August was 0.7 cm/day.

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REASSESSMENT OF *SIMONSENIA* (BACILLARIOPHYCEAE) FROM MORPHOLOGY AND MOLECULAR PHYLOGENETIC DATA SUGGESTS A RELATIONSHIP TO *PSEUDONITZSCHIA*

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Species belonging in the genus *Simonsenia*, including *S. delognei*, have been studied by means of light and electron microscopy. One of them, from estuarine environments in the Guadiana River (Atlantic coast, close to the border of southern Portugal) has also been maintained in culture, making it possible to use molecular (rbcL gene) data to study its relationships. In the LM, *Simonsenia* presents *Nitzschia*-like frustules with transapical costae being separated by the striae. It is only electron microscope (EM) examination that reveals that the raphe canal bearing keel is raised above the valve surface on alar canals, with the latter separated by fenestrae. Based on this,

Simonsenia has been proposed to be a genus intermediate between Bacillariaceae and Surirellaceae. However, the results of our study reveal that *Simonsenia* shares its major characteristics with *Nitzschia* (plastids) and *Pseudonitzschia* (plastids, striation, rbcL phylogeny). As in *Nitzschia*, there are two plastids in *Simonsenia*, one located towards each valve end. In EM part or all of the *Simonsenia* striae contain 2–3 transapical rows of areolae arranged in a quincunx pattern, strongly resembling the striation of *Pseudonitzschia*. Also the areolae occlusions are of the same type in both genera. Finally the rbcL gene sequence of the Guadiana River species seems to place *Simonsenia* in the same clade as *Pseudonitzschia*, *Denticula* and *Fragilariopsis* well separated from all remaining Bacillariaceae. Our study also shows that diversity of *Simonsenia* is probably underestimated, particularly concerning the marine environment because apart from the two established species, we have identified at least three unknown species from Portugal, Martinique and South Africa.

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TRAPS OF CARNIVOROUS PLANTS AS ALGAE MICROHABITATS

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Microhabitats are small ecosystems usually occupying closely separated areas with specific physical and chemical properties which often present a barrier to the growth of certain organisms or allow the development of only a small group of organisms. The aim of this study was to examine the structure of algae communities and their stability inside the traps of carnivorous plant taxa belonging to the genera *Utricularia* L. and *Genlisea* A.St.-Hil. Their traps constitute specific microhabitats. In the investigated *Genlisea* traps, two dominant groups of algae (*Chlamydomonas* div. and diatoms) in the trap environment showed an ability to hydrolyze phosphomonoesters. In the *Utricularia* traps, the algal flora derived mainly from the phytoplankton of the plants' environment, and its abundance increased consistently with trap age. The predominant organisms in the traps were either commensals or intruders, and occurred in numbers exceeding the captured macroscopic prey. We suggest that the fate of some

caught algal organisms most likely differs between the aquatic traps of different carnivorous plant species.

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A NEW CANDIDATE SPECIES OF CRASPEDOCARPUS (CYTOCLONACEAE, GIGARTINALES) FROM KOREA

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Seven species of *Craspedocarpus* (Cystocloniaceae, Gigartinales) have been reported from worldwide; five species from Australia and New Zealand, one from North Carolina, and one from Brazil. Our *Craspedocarpus* species collected from Jindo, Korea in August 2010 and June 2013. It was recognized by several morphological characters such as well developed rosettes of small outer cortical cells around the large inner cells, 3-5 cm in height, dichotomous branch, discoid holdfast, cystocarp produced from marginal thalli, zonate tetrasporangia and the terminal position of tetrasporangia. Phylogenetic analyses of rbcL indicate that our unidentified species is placed in genus *Craspedocarpus* and is also distinguishable from other *Craspedocarpus* species.

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LOCAL FRESHWATER MICROALGAE, SCENEDESMUS QUADRICAUDA, FOR NUTRIENT REMOVAL AND BIOFUEL PRODUCTION IN DIFFERENT WASTEWATER

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This article reports the results of the freshwater microalgae, *Scenedesmus quadricauda*, capable of removing nitrogen & phosphorus while accumulating lipids in primary settled & filtrate wastewater. For analysis of cell growth, optical density (OD), dry weight (DW) & cell number were measured. OD was measured at 1.36 units in both acclimated & un-acclimated cell after 16 days. The maximum value of DW in acclimated & un-acclimated cell was 0.995 g/L & 0.940 g/L after 14 days respectively. Due to cell adaptation in acclimation, it had positive growth rate

while the un-acclimated one had negative result in Day 1. The greatest daily growth rate of acclimated & un-acclimated cell was in Day 3 & Day 5 respectively. For nutrient analysis, 95% and 90% of P-pollutant, e.g. (PO₄-P), in acclimated & un-acclimated cell was removed after 5 days respectively. Over 90% of N-pollutants, e.g. NH₃-N, NO₃-N & NO₂-N, in both acclimated & un-acclimated cell was removed after 5 days. The lipid content of acclimated & un-acclimated cell was 20.3% & 19.8% respectively after 16 days. The lipid production was the highest in Day 3, then reached the lowest in Day 11 & increased until Day 14 in both conditions. The saturated fatty acid was higher in un-acclimated (~58%) than acclimated (~54%), while the poly-saturated fatty acid was higher in acclimated (~40%) than un-accumulated (~37%). Moreover, the cell growth in 25% & 50% filtrate was higher than in 75% & 100% ones. Due to higher N concentration in filtrate, cell had positive daily growth rate after Day 3. The removal efficiency of N & P was higher in 25% and 50% filtrate. For lipid production, the highest one was in 50% filtrate (18.1%) while the lowest was in 100% filtrate (13.6%). So it is an innovative tool for wastewater treatment & biofuel production so as to mitigate the red tide formation and create a green energy for future use.

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DEVELOPING OPTIMIZED CULTURE MEDIA FROM WASTEWATERS FOR THE EMERGING MICROALGAE-BASED BIOFUEL INDUSTRY

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Agricultural runoff, cattle, swine, poultry, and municipal wastewaters were aerobically digested and evaluated for their performance as a culture medium in comparison to the algae industry standard BG-11 culture medium. Preliminary data were gathered on the digestion of the wastewaters to track nutrient release from solid waste. Nitric acid digestion and ICP analysis showed that the wastewaters generally contained most of the nutrients necessary to sustain microalgae growth. Aerobic digestions carried out on the wastewaters showed a significant release of nutrients via ICP and flow injection analysis, although

the type and quantity of nutrients released differed significantly with different wastewaters. Municipal wastewater shows the most promise by demonstrating a rapid release in nutrients and exhibiting relatively little propensity for contamination, while cutting the cost of culture media in half when compared to BG-11. Likewise, cultures of *Scenedesmus sp. 0414* cultivated on municipal wastewater media exhibited similar growth and lipid productivities as cultures grown on BG-11. A similar process is underway to evaluate several local agricultural waste streams for their commercial potential as algae culture media.

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SPLICE-RELATED CODON AND AMINO SKEWS AT EXONIC ENDS IN ECTOCARPUS ARE COMMON AND RESEMBLE THOSE SEEN IN HUMANS

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The control of RNA splicing is often modulated by exonic splice enhancers (ESEs), causing predictable skews in codon and amino acid usage towards exonic ends, especially in species with relatively abundant introns. To date the only intron-rich species that have been analysed are mammals, precluding any conclusions about the likely ancestral condition. Here we examined the patterns in the vicinity of exon-intron junctions in *Ectocarpus siliculosus*. Noteworthy findings were: (1) amino acids and codons preferred/avoided at both 3' and 5' ends tended to also be preferred/avoided at the same exon ends in humans; (2) the preferences observed at the 5' ends of exons were largely the same as those at the 3' ends, a symmetry trend only previously observed in animals; (3) the proportion of codons and amino acids showing a significant skew towards exon ends was greatly in excess of anything reported previously. We conclude that: (a) in intron rich genomes splice associated skews are highly phylogenetically conserved; (b) symmetrical exon trends are not restricted to animals; and (c) the exclusivity of translationally optimal and splice optimal codon sets is not universal.

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ELEVATED CO₂ IN COMBINATION WITH INCREASED TEMPERATURE – EFFECTS ON SPRING AND SUMMER PHYTOPLANKTON COMMUNITIES

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Due to the complexity of environmental factors associated with climate change, and their potential synergistic effects, we believe that a multifactorial approach is required to resolve and predict climate effects on phytoplankton communities. Therefore, we tested the effects of elevated CO₂ and temperature on a spring and summer phytoplankton community, respectively. The experiments were conducted in collaboration with the KOSMOS 2013 mesocosm experiment in the Gullmar Fjord inlet at the Swedish west coast. Target levels of pCO₂ were 400 and 1000 μatm. The spring community was exposed for the target pCO₂ for 6 weeks and the summer community for 12 weeks before we applied a temperature gradient of five temperatures. For the spring community, preliminary results show that photosynthetic activity (Fv/Fm) increased with temperature and CO₂ had no effects. For particulate primary productivity, significant treatment effects were found for both temperature and CO₂ treatments; the ambient temperature differed from the two highest temperatures and primary productivity was higher in elevated CO₂. Species composition, biomass, bacterial biomass and productivity remain to be analyzed. Results from the summer community experiment have not yet been compiled.

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GLUCOSE MODULATES PHOTOSYNTHETIC ACTIVITY AND INDUCES SYMBIODINIUM BLEACHING

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The cnidarian-dinoflagellate mutualism is essential for the survival of the coral-reef ecosystem. Despite the enormous ecological and economic importance of

corals, their cellular and molecular biology is still poorly understood. We have been developing a proxy system for examining coral mutualism in which an axenic dinoflagellate symbiont *Symbiodinium* is introduced into a clonal population of the host *Aiptasia*, a small sea anemone closely related to corals. Axenic *Symbiodinium* strain SSB01 is being examined for both its growth and physiological characteristics. When cultured in low light with glucose, the cells lose pigmentation and photosynthetic activity. However, in spite of the loss of pigmentation, the algae maintain rapid heterotrophic growth. When bleached cultures are moved into medium without glucose, the cells regain their pigmentation, photosynthetic activity and the capacity to grow photoautotrophically. We are currently using RNA-seq to characterize global gene expression in SSB01 during both the bleaching and recovery process. Our studies will provide an entry point into elucidation of chloroplast biogenesis, pathways for heterotrophic and photoautotrophic growth in dinoflagellates, and generate insights into mechanisms associated with coral bleaching.

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ALLELOPATHIC EFFECTS OF THE MARINE DIATOMS *PSEUDO-NITZSCHIA MULTISERIES* AND *PSEUDO-NITZSCHIA PUNGENS* ON COMPETING PHYTOPLANKTON

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Here we demonstrate the ability of three strains of *Pseudo-nitzschia* isolated from the South China Sea and the Bay of Fundy, Canada, to produce extracellular compounds capable of lysing and/or inhibiting the growth of three phytoplankton species during bi-algal culture experiments. Since extracellular, lytic activity was found for both *P. multiseriis*, which produces domoic acid (DA), and *P. pungens*, which produces little if any DA, the allelopathic effects of *Pseudo-nitzschia* spp. seem to be unrelated to DA. Allelopathic inhibition of other phytoplankton was documented during exponential and stationary phase growth of *Pseudo-nitzschia* cells and the strongest allelopathic effects were obtained

from sonicated cultures, suggesting the sudden release of allelochemicals via processes such as cell lysis or zooplankton grazing may have the strongest effect in an ecosystem setting. Differences in the responses of target species to *Pseudo-nitzschia* spp. suggest these algae may produce multiple compounds that vary in their allelopathic potency and composition as a function of species, strain, growth stage, and perhaps other factors. Allelopathy may play important role in the formation and persistence of natural and iron-fertilized *Pseudo-nitzschia* blooms.

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DIVERSITY OF MORPHOLOGY AND PHOTOSYNTHETIC PIGMENTS IN DINOTOMS, INCLUDING THOSE OF TWO NEW SPECIES

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Currently, 11 species of ‘dinotom’ (=dinoflagellates harboring a diatom endosymbiont) are known. We successfully cultured 6 ‘dinotoms’ including two undescribed species (strains DA10 and DA11), and analyzed their photosynthetic pigment profiles by HPLC in addition to taxonomic studies for two new species. Free-floating non-motile cell clumps are dominant throughout the division cycles of the two new species. Each cell is almost spherical and the cell surface of DA10 is verrucose, while that of the DA11 is smooth. Both are close to *Galeidinium rugatum* based on SSU rDNA phylogeny, which can be distinguished by having attached and dome-like non-motile cell with a wrinkled surface. A total of 22 unknown carotenoids were detected in all 6 ‘dinotoms’. DA10 and *G. rugatum* share 9 unknown but unique carotenoids, so this suggests *G. rugatum* is more close to DA10 than to DA11. Surprisingly, 19 out of the 22 unknown carotenoids are not shared with those of the free-living diatom, *Cylindrotheca closterium*, which is phylogenetically close to ‘dinotom’ endosymbionts. This indicates that ‘dinotoms’ acquired unique carotenoids after gaining a diatom as the plastid.

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CELL DIVISION CYCLE AND DISCHARGE PATTERN OF A SYMBIOTIC DINOFLAGELLATE SYMBIODINIUM WITHIN ACROPORA CORAL

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Corals utilize photosynthetic products of symbiotic dinoflagellate *Symbiodinium* spp. Although *Symbiodinium* discharge can take place under non-stress conditions, little is known about the timing or quantity of cells discharged. We set up an experiment in which coral *Acropora muricata* was maintained in 12-L aquariums with running seawater (27 °C), under LED lights in a 12 h light:12 h dark cycle (07:00–19:00 light and 19:00–07:00 dark). Aquarium water samples were collected every 3 h to quantify discharged planktonic *Symbiodinium* cells, using quantitative real-time PCR. In addition, symbiont cells remaining within the corals were observed under microscope, to determine the cell division cycle. Discharge of *Symbiodinium* cells reached a peak, of ca. 460000 cells from 196 g (skeletal weight) coral, during the 10:00–11:00 period. *Symbiodinium* cell divisions were synchronous, reaching a peak at 04:00–05:00. The cell division pattern was congruent with that of cultured *Symbiodinium*, indicating that this cycle is not completely disturbed in individuals living symbiotically within corals. The proliferated *Symbiodinium* cells were discharged and/or digested by corals, which served to adjust the algal density within corals.

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SPECIES DIVERSITY OF GIGARTINALES (RHODOPHYTA) WITH THE NEW DISCOVERY OF HYPNEA AND CRASPEDOCARPUS

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The order Gigartinales contains red algae of the class Florideophyceae. It is considered a large, unwieldy, and complicated order, an artificial construct formed by merging the Cryptonemiales and Gigartinales.

Much progress and effort have been made in the last decade to resolve the complexity of relationships among members in this order. In Korea, 24 genera and 62 species of Gigartinales are reported in the literature. Although several species have economic importance, the order has never been studied to determine its accurate taxonomic status in Korea. We started the survey of Korean Gigartinales to confirm how many species are present, to determine the phylogenetic relationships among them, and to discover cryptic species. We analyzed nuclear SSU, plastid *rbcL*, and mitochondrial COI genes for molecular phylogeny. As a result, so far we have identified 11 families, 15 genera and 30 species in the order. Among them, two species of *Hypnea* have the potential to be a new species and the genus *Craspedocarpus*, typically distributed in the southern hemisphere, is found for the first time in Korea. The research is progressing to identify the proper scientific names demonstrated by molecular support and redefining morphologically confused taxa. The case study presented here will further enhance our knowledge of systematics including cryptic species in the order Gigartinales.

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EFFECTS OF LIGHT QUALITY ON ETTLIA OLEOABUNDANS: ROLE OF RED AND FAR-RED LIGHTS ON GROWTH AND OIL

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The effect of light quality on biomass and lipid production was investigated in *Ettlia oleoabundans*. Previous studies showed that cultures under full spectrum light produced significantly more triacylglycerides compared to cool white, which emits little red or far-red. Compared to cool white controls, and keeping total light intensity constant, when red was added at day 14, maximum biomass was 1.6-1.8 g L⁻¹ for both conditions, but triacylglycerides increased 2.5-fold when the red to cool white fluence ratio was 1:1. On the other hand, triacylglyceride yields decreased when the proportion of red was reduced, or by adding far-red when red was already present. If only far-red was added to cool white, there was no significant increase in triacylglycerides. Furthermore, while adding red decreased palmitic, oleic acid

increased; oleic is a desired biodiesel precursor. Longer exposure to red further enhanced oleic acid content per cell. While this study showed that red light, but not necessarily far-red, can improve triacylglyceride yield and quality in *E. oleoabundans*, further study into red far-red switching should prove useful for this and other oleaginous algal species.

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SEAWEED AQUACULTURE FOR NUTRIENT BIOEXTRACTION IN LONG ISLAND SOUND AND THE URBANIZED BRONX RIVER ESTUARIES

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Gracilaria tikvahiae and *Saccharina latissima* were cultivated and harvested for nutrient bioextraction at two sites: off Fairfield, CT (LIS), and the other at the mouth of the Bronx River estuary (BRE) during 2011-2013. *Gracilaria* grew up to 16.5% d⁻¹ at the BRE site. *Saccharina* grew from <1mm to 3.0 m in length and yielded 18 kg m⁻¹ of longline over the five months (Dec.-May) of the growing season. We estimated a hypothetical one hectare farm of *Gracilaria* at the LIS site, with 3 m spacing between longlines, could remove 5.0 (Aug.) to 7.9 (Oct.) kg N ha⁻¹ mon⁻¹, and 9.96 (Oct.) to 41.9 (July) kg N ha⁻¹ mon⁻¹ at the BRE site per hectare. *Saccharina*, with 1.5 or 6 m spacing between longlines, could remove 71 to 280 kg N ha⁻¹ during that winter-spring growing season at our hypothetical nutrient bioextraction farm in LIS. These results suggest that year round seaweed aquaculture can be a useful technique for nutrient bioextraction in urbanized coastal waters. The potential applications using the harvested *Gracilaria* and *Saccharina* were also tested for human food, hydrocolloids, animal feed, and biofuel.

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MICROPROPAGATION OF MALAYSIAN ECONOMICALLY IMPORTANT SEAWEEDS FOR THE PRODUCTION OF CLONAL PLANTING MATERIALS

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Good quality seedlings for mariculture of commercial seaweeds ensure sustainable production for various applications including food and industrial colloids. Tissue and protoplast culture was conducted on *Kappaphycus*, *Gracilaria* and *Ulva*. Agar concentration, growth hormones, irradiance and enrichment media were used to optimise culture conditions for callus induction and regeneration. The first filamentous callus on *Kappaphycus* was observed six days after the explants were cultured in agar solidified PES medium and more than 95% of the cultured explants produced calli after 30 days. Filamentous and compact calli were induced at the cut surface of the explants and derived from both cortical and medullary cells. Regeneration of calli was observed. Filamentous callus was observed after 16 days for *Ulva*. Protoplast were produced and regenerated into plants after three months for *Gracilaria changii*. This suggested the possible use of micropropagation technique as an alternative propagation technique for seedling production of economically important seaweeds.

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REDTOL: RED ALGAL TREE OF LIFE

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Red algae (Rhodophyta) are comprised of more than 6000 species classified into seven classes. They play an important role in marine and freshwater ecosystem as primary producers, and they are directly used as foods and industrial usage as carrageenan and agar production. This anciently diverged eukaryotic phylum is regarded as a plastid donor for the photosynthetic chromalveolates. Despite evolutionary, ecological, and

economic importance, the red algal tree of life has been under-studied. To fill this gap in eukaryote phylogeny, we assembled a research team of eight red algal taxonomy and genomics experts to address fundamental questions in red algal evolution and their place in the ToL. Nine genes (*rbcL*, *psaA*, *psaB*, *psbA*, *cox1*, *cob*, *EF2*, *28S* and *18S rDNA*) were determined from ca. 500 taxa, and here we are presenting the taxon-rich phylogenies. We are conducting draft genomes sequencing from 16 taxa. As a preliminary result, we are introducing dozens of new mitochondrial genomes and a data-rich phylogeny with an evolutionary interpretation.

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ENGINEERING AND COMMERCIAL ASPECTS OF ALGAE-BASED WASTEWATER TREATMENT TO PREVENT COASTAL DEADZONES

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A major source of nutrients that cause algal blooms and dead zones in coastal waters is inadequately treated municipal wastewater. Although EPA launched a national nutrient removal program in 1998, the majority of 16,600 operating plants in U.S. are still ill-equipped to remove nutrients. EPA has established national water quality standards for nutrients, and developed pollution load allocation through the Total Maximum Daily Load program. Although technologies exist for removing nutrients, they tend to be high in capital and operating cost. An alternative to deploying existing technologies is to integrate algae cultivation into the wastewater treatment process to take advantage of alga's inherent capacity to uptake nutrients. Historically, algae have been incorporated into pond treatment systems serving small communities. More robust systems are needed that can serve population centers. Residual biomass from such algae-based wastewater treatment can serve as feedstock for production of marketable products. The combination of nutrient removal and production of marketable products presents a unique opportunity for the development of a public/private commercial initiative. Such an effort represents one approach to begin addressing coastal dead zones.

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CULTIVATION OF NATURAL ALGAL ASSEMBLAGES FOR WASTEWATER NUTRIENT REMEDIATION

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While stimulation of algal growth by eutrophication of coastal waters contributes to dead zones, algae can also offer opportunities for mitigating terrestrial nutrient inputs to freshwater resources and marine coastal waters. As well as remediation of nutrient loadings in effluent waters, algal biomass produced can be used as feedstocks for biofuels. Growth of algae for biofuels is not new but traditional approaches using unialgal cultures in 'bioreactors' present challenges for culture maintenance, cell harvesting and energy and clean water demands. Engineering life cycle comparisons indicate that algal cultivation in wastewater can offset costs and environmental burdens of algal biomass production over broader climatic regimes. Using nutrient-rich wastewater, we have been growing natural algal assemblages dominated by cellulose-rich filamentous green algae and lipid-rich diatoms, which do well in a cool temperate climate. We will present data on nutrient removal characteristics, lipid and cellulose production and seasonal biomass harvesting in wastewater cultivation. We will describe a strategy for using algal cellulose as a feedstock for bacteria genetically-modified to produce specific and uniform biofuel substrates.

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TESTING THE UTILITY OF RED SEA MARINE DIATOMS AS MODEL ORGANISMS FOR BIOTECH APPLICATIONS

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This project seeks to test the utility of marine diatoms from the Red Sea as model organisms for biotechnology. Our labs are responsible for completing four objectives: survey the Red Sea diatom flora and

establish cultures, assay the cultures to find strains with high-lipid content, sequence plastid genomes for 10 high-lipid content diatoms, and transform the plastid of one of these species. A January 2013 collecting trip to the Red Sea has so far yielded 112 strains identified to genus (52 of which are pennate diatoms), and these have been added to our existing collection of over 100 marine diatom strains from other locations. Total lipid has been extracted from more than 30 strains, and lipid content was measured by thin layer chromatography. The plastid genome of a marine diatom *Cerataulina daemon* has been completed and annotated. The genome is 120,144 basepairs with an inverted repeat of 7,004 basepairs. Preliminary analyses indicate that protein-coding gene contents are nearly identical to those from *Phaeodactylum tricornutum* but gene order is rearranged.

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MULTIPLE BIO-PRODUCTS FROM A NOVEL MARINE DIATOM *ODONTELLA AURITA* (BACILLARIOPHYCEAE)

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Odontella aurita is a unicellular marine diatom containing multiple bioactive compounds. The maximum biomass concentration obtained in batch cultures with modified L1 medium was up to 6.0 g L⁻¹. The glycolipids were dominating lipid class in *O. aurita*, and its proportion was significantly higher than the neutral lipids and phospholipids. The major fatty acid compositions of *O. aurita* were C14:0, C16:0, C16:1, and C20:5 (EPA). EPA was mainly deposited in the glycolipids. The main pigment compositions of *O. aurita* were composed of chlorophyll *a*, chlorophyll *c*, fucoxanthin, diadinoxanthin, diatoxanthin, and β -carotene. High purity of fucoxanthin (>97%) was obtained from *O. aurita* cultures, which was further identified as all-trans fucoxanthin. The chrysolaminaran was found to be the major storage carbohydrate in *O. aurita*, and its content could reach as high as more than 50% of dry weight. The purified chrysolaminaran was mainly composed with glucose and mannose, and it was further identified as β -(1 \rightarrow 3), (1 \rightarrow 6)-glucan. The purified fucoxanthin and chrysolaminaran exhibited certain antioxidant

activities by scavenging free radicals in a time- and dose-dependent manner.

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NEW MARINE SPECIES AND TOXINS FROM MARINE TEXAS WATERS

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At least six divisions of algae produce algal toxins. Our work has focused on identification of these toxins, and currently we are using physiological experimentation to understand mechanistic issues. Recent work on Texas blooms has focused on: 1) *Euglena sanguinea* and >12 species produce euglenophycin toxin in saline waters. Fish, invertebrates, and mammalian cell lines are killed from exposure. Euglenoids survived in motile states at 70 ppt salinity. 2) *Prymnesium parvum* produces fatty acid amides (FAA) that cause fish kills. FAAs are present in all strains tested, prymnesins are not. 3) A new *Geitlerinema* species from marine sediments produces a unique algal toxin having a mass of 475.3 amu. This toxin kills shrimp, mammalian cell lines, and fish. 4) A coccoid *Synechococcus*-like cyanobacteria produces a chloroform soluble toxin that is partially characterized. The toxin kills fish.

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APPLICATION OF FLUORESCENCE TECHNIQUES IN STUDIES OF FACTORS AFFECTING GROWTH AND VIABILITY OF NATURAL DIATOM POPULATIONS IN A EUTROPHIC FRESHWATER RESERVOIR

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We measured growth and viability of *Fragilaria crotonensis*, a dominant diatom of the summer phytoplankton in the eutrophic, dimictic freshwater Rimov Reservoir (Czech Republic). Over two

consecutive seasons (2011–12, June – September), *Fragilaria* growth and viability was assessed at weekly intervals using PDMPO, a specific Si-deposition tracer in diatoms, and SYTOX Green, a membrane-impermeable nucleic acid stain, respectively. Using multiple linear regression with stepwise forward selection of environmental variables, diatom growth and viability were found to be driven by different factors. *Fragilaria* growth was positively affected by daily light exposure ($r^2 = 0.52$, $p < 0.001$) and by mean river discharge into the reservoir ($r^2 = 0.34$, $p < 0.001$). However, *Fragilaria* viability declined markedly with decreased ambient silica concentration ($r^2 = 0.60$, $p < 0.001$), indicating that while diatom growth is tightly related to physical properties of the environment, cell viability reflects the availability of silica, which is essential for generating and maintaining diatom siliceous frustules. Interestingly, there was a significant negative correlation between *Fragilaria* viability and growth ($r^2 = 0.41$, $p < 0.001$), suggesting that seasonal peaks of diatom growth are coupled with low cell viability.

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RED ALGAL SPECIES: WHERE DO WE STAND, WHERE DO WE GO?

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Molecular sequence data has revealed a large amount of genetic variation within many morphologically described species. This data is often partitioned into clusters of samples occurring sympatrically or allopatrically, based on sequence divergence and tree topology. While such cryptic species are being continually recognized, it is commonly unclear if these lineages fulfil other criteria of species. An important aspect of species distinction has always been reproductive isolation. While the genetic basis of speciation is poorly understood in red algae, the potential of reproductive compatibility may render these species short-lived once sympatry is established. Research shows that reproductive isolation is commonly reflected in sequence divergence, but also that hybridization can occur between genetic clusters and that reproductive incompatibility can occur within clusters. Thus, the validity of these two concepts, that reproductive isolation defines species and that lineages are species, needs to be backed up with more data and

targeted research. While red algae produce propagules that can disperse, it has become clear that these events are rare in most taxa and that populations can be isolated even at short distances. Reduced gene flow, together with natural selection, is a driving force of speciation. However, we have only begun to appreciate that isolation can be accompanied by fixed physiological changes. Combining molecular species designation with ecophysiological studies should be a focus to further our understanding of the selective forces that generate and shape red algal diversity. The examples I will use will be drawn mostly from my work in mangrove algae of the genera *Bostrychia* and *Caloglossa*.

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