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## TAXON SAMPLING AND INFERENCES ABOUT DIATOM PHYLOGENY

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Proper taxon sampling is one of the greatest challenges to understanding phylogenetic relationships, perhaps as important as choice of optimality criterion or data type. This has been demonstrated in diatoms where centric diatoms may either be strongly supported as monophyletic or paraphyletic when analyzing SSU rDNA sequences using the same optimality criterion. The effect of ingroup and outgroup taxon sampling on relationships of diatoms is explored for diatoms as a whole and for the order Thalassiosirales. In the latter case, SSU rDNA and rbcL sequence data result in phylogenetic relationships that appear to be strongly incongruent with morphology and broadly incongruent with the fossil record. For example, *Cyclotella stelligera* Cleve & Grunow behaves like a rogue taxon, jumping from place to place throughout the tree. Morphological data place *C. stelligera* near the base of the freshwater group as sister to the extinct genus *Mesodictyon* Theriot and Bradbury, suggesting that it is an old, long branch that might be expected to “misbehave” in poorly sampled trees. *Cyclotella stelligera* and *C. bodanica* Grunow delimit the diameter of morphological diversity in *Cyclotella*, so increased sampling of intermediate taxa will be critical to resolving this part of the tree. Morphology is sampled for a much greater number of taxa and many transitional states of putative synapomorphies seem to suggest a robust morphological hypothesis. The Thalassiosirales are unstable with regards to taxon sampling in the genetic data, suggesting that perhaps the morphological hypothesis is (for now) preferable.

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## INFLUENCE OF MIXED HOST POPULATIONS ON SUCCESS OF THE PARASITIC DINOFLAGELLATE AMOEBOPHRYA

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Parasitic dinoflagellates of the genus *Amoebophrya* commonly infect bloom-forming dinoflagellates of Chesapeake Bay, including *Akashiwo sanguinea* and *Karlodinium micrum*. While different strains of *Amoebophrya* appear host specific, infective dinospores liberated from *A. sanguinea* do enter the cytoplasm

of *K. micrum*, but fail to complete the infection cycle. Thus, in mixed-species dinoflagellate blooms, interference from inappropriate hosts may influence the success of *Amoebophrya* spp. To explore that possibility, we conducted laboratory studies to examine the effect of the toxic dinoflagellate *K. micrum* on success of *Amoebophrya* from *A. sanguinea*. Treatments consisted of *A. sanguinea* (1000/mL) plus corresponding dinospores (10,000/mL) in the presence of different *K. micrum* densities (0 to 100,000/mL). We also examined whether changes in parasite success were due to interaction with *K. micrum* cells, or from indirect effects of bacteria or dissolved substances present in *K. micrum* cultures. Success of *Amoebophrya* was unaffected by low densities of *K. micrum*, but decreased at high concentrations of *K. micrum*. Reduced parasite success appeared to result from combined effects of non-host cells and dissolved substances in *K. micrum* media. Results suggest that parasitism of *A. sanguinea* in Chesapeake Bay would be reduced when *K. micrum* is a major component of mixed-species blooms.

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## PHYLOGENETIC AFFINITY OF THE PALMELLOID GREEN ALGAE, VERDIGELLAS AND PALMOPHYLLUM (CHLOROPHYTA), BASED ON ANALYSES OF NUCLEAR-ENCODED SMALL SUBUNIT rDNA SEQUENCES

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Palmophyllum, Verdiggellas and Palmocladus are marine palmelloid green algae with morphologies ranging from closely adherent crusts, peltate discs, to upright branched thalli. Thalli of these taxa are comprised of small spherical cells embedded within a dense mucilaginous matrix. Taxonomic affinities of these palmelloid genera, however, has remained uncertain. Previous studies of Palmophyllum and Verdiggellas classified these algae within the Palmellaceae, but the complete absence of data regarding reproduction have blurred ordinal designations. Generally, these algae have been classified as members of the Tetrasporales within the class Chlorophyceae, but the Chlorococcales has also been proposed. Global analyses of eukaryotic nuclear-encoded small subunit rDNA sequences based on parsimony (MP),

neighbor joining (NJ) and likelihood (ML) methods confirm the placement of *Palmophyllum* and *Verdigellas* as a monophyletic group within the Chlorophyta, but class and ordinal affinities were not clearly resolved. ML suggested that *Verdigellas* and *Palmophyllum* are members of a clade with coccooid prasinophyte algae at the base of the Chlorophyta, while NJ and ML suggested that the palmelloid genera formed a basal lineage of the Viridiplantae. A consistent feature of all analyses, however, is that *Verdigellas* and *Palmophyllum* did not group with the chlorophycean orders, Tetrasporales or Chlorococcales. Results will be discussed in the context of taxonomy, character evolution, and implications for green plant evolution. (Supported in part by NSF grants DEB-0128952 to MWF, DEB-0129030 to MAB, and DEB-0128977 to FWZ)

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**PHYLOGENY OF THE DASYCLADALES (ULVOPHYCEAE, CHLOROPHYTA) BASED ON ANALYSES OF NUCLEAR-ENCODED LARGE SUBUNIT RDNA**

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The Dasycladales is an ancient order of tropical benthic marine green algae, unique in their radially arranged unicellular thalli and well-preserved fossil record due to extensive calcification of the thallus. The inference of an accurate phylogeny for the Dasycladales is important in order to better understand stratigraphy, character evolution, and classification. Previous analyses (*rbcL* and 18S rDNA) suggested that the Family Acetabulariaceae is monophyletic, but that the Family Dasycladaceae is a basal paraphyletic assemblage. However, the two data sets disagreed regarding genus- and species-level relationships within the Dasycladales. For example, the placement of the genera, *Halicoryne*, *Bornetella* and *Cymopolia* were incongruent. Given the conflicting results of these previous analyses, the current project examined a third highly conserved nuclear-encoded gene, 26S rDNA. Aligned 26S rDNA sequences were analyzed with parsimony and model-based methods and compared to previous results based on 18S and *rbcL* sequences. Family-level relationships based on 26S rDNA were congruent with previous studies: the Acetabulariaceae is monophyletic while the Dasycladaceae is paraphyletic. In addition, acetabulariacean genera are not monophyletic, suggesting that the presence of a corona inferior or calcification of gametes may not be appropriate to define genera. Within the Dasycladaceae, the basal position of *Cymopolia* is supported by 26S rDNA, a result congruent with *rbcL* and stratigraphy but not with 18S data. These results will be discussed in the context of morphological character evolution, fossil

stratigraphy and family, tribal and generic relationships among these living algal fossils. Supported in part by NSF grant DEB-0128977 to FWZ.

## 5

**THE ORIGIN OF THE DINOFLAGELLATE PLASTID**

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Dinoflagellates are griffins, that is the merger of two eukaryotes: a heterotrophic host, the dinoflagellate, and a photosynthetic eukaryote. Our work has been focussed on the endosymbiont's identity and the massive gene transfer implied by secondary endosymbiosis. Dinoflagellates have many different types of chloroplasts, but the majority of photosynthetic dinoflagellates are pigmented with the carotenoid peridinin and chlorophyll *c*. The chloroplast genome of these organisms is cryptic. Genes that have been attributed to the chloroplast are encoded on minicircles that contain one or two genes. Phylogenetic analysis using these genes suggests that the peridinin chloroplast was derived from haptophytes. Since these minicircle genes have an extreme rate of change phylogenetic methods may produce misleading results. Statistical tests were used to test the quality of phylogenetic methods given these data, particularly the maximum likelihood model. To find plastid associated genes with more reasonable evolutionary rates an expressed sequence tag survey was begun. Among these sequences many typically plastid encoded genes were found including six genes that are exclusively plastid encoded in other eukaryotes. These data suggest massive chloroplast to nucleus gene transfer in dinoflagellates, and fits well with the small complement of minicircle genes. Phylogenetic analyses using these typically plastid encoded genes was also performed. It is now possible to directly test hypotheses of plastid origin within dinoflagellates and the chlorophyll *c* containing algae.

## 6

**DOES TEMPERATURE RESTRICT THE LATITUDINAL DISTRIBUTION OF SYMBIOTIC ZOOXANTHELLAE WITHIN THE SEA ANEMONE *ANTHOPLLEURA ELEGANTISSIMA*? EXPERIMENTS WITH *SYMBIODINIUM CALIFORNIUM* IN CULTURE**

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The intertidal anemone *Anthopleura elegantissima* hosts two species of symbiotic zooxanthellae with different latitudinal distributions within the anemone host. One of these species, *Symbiodinium muscatinei*, occurs in anemones from southern California to Washington, while the other species, *Symbiodinium californium*, is restricted to California and is not found in Oregon or Washington. To investigate if temperature restricts the latitudinal distribution of *S. californium*, thermal optima and limits to growth and photosynthesis were determined by culturing zooxanthellae at a range of temperatures (5–30 C). Mean growth rates ( $r$ ) were highest between 15 and 28 C (0.21–0.26 d<sup>-1</sup>) and extremely low at 5, 10, and 30 C (0.02–0.03 d<sup>-1</sup>). Light-saturated maximum photosynthesis ( $P_{max}$ ) increased with temperature between 20 and 30 C (2.93 to 13.18 pg C cell<sup>-1</sup> · h<sup>-1</sup>, respectively). The effect of temperature on cell motility and cell volume was assessed by culturing zooxanthellae at 10, 20, and 30 and determining % motility over a 24-hour period. Cell volumes were also calculated. No apparent change in % motility was observed with respect to time of day. Percent motility and cell volumes were greatest at 10 C (60% and 820 mm<sup>3</sup>, respectively). *S. californium* exhibited a dramatic reduction in growth (–89%) and photosynthesis (–64%) at temperatures within the range of average coastal sea temperatures in Oregon and Washington (8–17 C) than at higher temperatures (20–30 C). The results strongly suggest that the latitudinal distribution of *S. californium* is restricted by its reduced ability to grow or photosynthesize at low temperatures. Comparative studies exploring thermal thresholds of *S. muscatinei* are needed to conclusively demonstrate the role of temperature in determining the latitudinal distribution of both zooxanthellae in *A. elegantissima*.

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**INTEGRATING AUTONOMOUS DATA ACQUISITION AND FORECASTING INTO REGIONAL MONITORING EFFORTS; A SYNOPSIS OF THE MERHAB 2002: EASTERN GULF OF MEXICO SENTINEL PROGRAM**

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Blooms of the red-tide dinoflagellate, *Karenia brevis*, occur annually in coastal waters of the eastern Gulf of Mexico (GOMx) and impact marine resources, public health, and community economics. Early forecasting of and subsequent mitigation for blooms are objectives of agency, academic, and private partnerships. The 5-year, NOAA-funded MERHAB 2002: Eastern GOMx Sentinel Program will develop and assess the utility of a networked system of autonomous sampling platforms. The goal of the program is to become a strategic, proactive hardware- and information-technology for operational GOMx coastal observatories and as such, facilitate model initializations and state-wide, adaptive field sampling. Monitoring platforms will utilize both existing and newly established buoys, and autonomous, water-column profiling vehicles incorporating physical, chemical, and biological sensors. The biological sensors, including a bio-optical phytoplankton discriminator and a processor containing a molecular-probe array, previously have provided consistent results in laboratory and field trials and show great promise for remote, autonomous discrimination of *K. brevis*. Generated data will initialize a coupled bio-physical forecast model and validate remote sensing algorithms for *K. brevis* blooms. Data acquisition and forecasting efforts will be integrated with a geographically-comprehensive, rapid response component that incorporates adaptive, field sampling and a volunteer sampling network. NOAA's National Coastal Data Development Center will manage instrument data streams and disseminate and integrate all data into existing (and future) coastal partnerships with their associated management and communication network.

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**GRAZING BY HETEROTROPHIC NANOFLAGELLATES ON VIRUS- AND BACTERIA-SIZED PARTICLES IN TWO LAKES OF DIFFERENT TROPHY**

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Although viruses are now widely recognized to infect aquatic bacteria as well as prokaryotic and eukaryotic phytoplankton, our understanding of how they fit into aquatic foodwebs remains still deficient. The present study examined the potential nutritional food source that viral particles represent for natural assemblages of heterotrophic nanoflagellates (HNF). We determined the seasonal and depth-related variability in the grazing activity of HNF on virus- and bacteria-sized particles in the oligomesotrophic Lake Pavin and the eutrophic Lake Aydat. Ingestion rates were determined using 50 and 500 nm diameter fluorescent microspheres. Estimated ingestion rates

ranged from 0.01 to 1.7 viruses cell<sup>-1</sup>·h<sup>-1</sup> (Aydat) and from 0.01 to 2 viruses cell<sup>-1</sup>·h<sup>-1</sup> (Pavin). Derived clearance rates for viruses represented 3–5% (Aydat) and 10–12% (Pavin) of those for bacteria. In general, HNF grazing on both viruses and bacteria showed a similar pattern characterized by an apparent decrease in summer followed by autumnal peaks, with lowest values always being recorded in the hypolimnion. Viral production, estimated from the frequency of visibly infected cells (using transmission electronic microscopy), was consumed by HNF at rates averaging 2% (Aydat) and 10% (Pavin). This study suggests that, viruses, which are assumed to correspond to 1 × 10<sup>-7</sup> ng C/particle, are likely to be of low nutritive value for HNF in both lakes. However, these results imply that HNF grazing may contribute significantly to the removal of viruses in freshwaters, especially in low productive systems.

## 9

**BEYOND BIOGEOGRAPHY: LARGE-SCALE PATTERNS OF DISTRIBUTION AND ABUNDANCE OF INTERTIDAL MARINE ALGAE ALONG THE US WEST COAST**

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Biogeographers have long been intrigued by the richness and diversity of the Northeast Pacific algal flora. Most studies have focused attention on distributional patterns delineated into provinces and most strongly influenced by temperature. However, biogeographical studies have always been plagued by the necessity of interpreting distributional data that may be incomplete, non-quantitative, limited in spatial extent and taxonomic resolution. We attempt to overcome some of these impediments by providing a quantitative description of intertidal community structure across a large spatial scale; ranging from northern Washington to southern California, including the California Channel Islands. We quantified patterns of distribution and abundance of marine algae and invertebrates in permanently marked grids extending 30 m along the shoreline and vertically from the upper barnacle zone to the surfgrass zone at MLLW. We identified most organisms to species level, when possible and collected voucher specimens of all taxa. Here we present the results of these studies focusing on geographic patterns of algal richness and diversity, patterns of abundance across geographical ranges, and functional group abundances relative to regional and mesoscale oceanographic patterns.

## 10

**CHLAMYDOMONAS ACIDOPHILA FROM THE BERKELEY PIT LAKE – A STRAIN ADAPTED TO AN EXTREME ENVIRONMENT**

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This research was directed to gather a better understanding about the unicellular alga *Chlamydomonas acidophila*, one of the 6 algal species found in the Berkeley Pit Lake. Berkeley Pit Lake is a flooded, abandoned pit mine with a pH of 2.7 and high metal concentrations. It has been found that the effective concentrations of metals that limit the growth of *C. acidophila* by 50% were 9.024 mg/L for Cu<sup>2+</sup> and 75.4 mg/L for Zn<sup>2+</sup>. We have been able to grow *C. acidophila* from Berkeley Pit samples at high densities in medium containing 15.36 mg/L Cu<sup>2+</sup> and 83.65 mg/L Zn<sup>2+</sup>. Moreover, this species is able to grow in nutrified Berkeley Pit water, which contains approximately 110 mg/L Cu<sup>2+</sup> and 323 mg/L of Zn<sup>2+</sup>. The hypothesis is that the species found in Berkeley Pit Lake represents a genetic strain adapted to high metal concentrations environments. A comparison between the American Type Culture Collection strain of *C. acidophila* and the strain collected from Berkeley Pit was made. Growth rate of the two strains in Bold Basil Medium, Modified Acid Medium and Berkeley Pit nutrified water were calculated and compared. Moreover, preliminary investigations of the genome of *C. acidophila* from the Berkeley Pit Lake were initiated.

## 11

**NITROGEN LOADING BY INVERTEBRATES INCREASES GROWTH AND DIVERSITY OF INTERTIDAL MACROALGAE**

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High-zone intertidal pools along the Oregon coast are a nutrient-poor habitat within a nutrient-replete upwelling ecosystem. However, many seaweed species occur in tide pools, especially when sessile invertebrates are present. By converting particulate nitrogen into ammonium, filter-feeding invertebrates can mediate the supply of nitrogen to seaweeds in tidepools. Invertebrates excrete substantial quantities of ammonium into tidepools, which seaweeds take up and assimilate as a nitrogen source. We investigated the ecological consequences of this local-scale nitrogen source by evaluating the influence of invertebrate-excreted ammonium on the growth and

diversity of seaweeds in high-intertidal pools. We measured the growth of a macroalga commonly found in high-intertidal pools, the red alga *Odonthalia floccosa* (Esp.) Falkenb., when mussels were present and absent. Over a 24-day tidal cycle, accumulation rates of inorganic nitrogen were 3 times higher, and rates of nitrogen uptake were 4.5 times higher, when mussels were present, resulting in 50% more seaweed growth. The number of macroalgal species increased with the rate of ammonium loading into tidepools as slow-growing species tolerant of low nitrogen availability were supplemented by fast-growing species with higher nitrogen requirements. Thus, especially in high-intertidal pools, which are isolated from oceanic nitrate inputs for nearly 80% of the time, invertebrates are important contributors to the nitrogen budgets of seaweeds. This study highlights the role of local-scale nutrient regeneration as a potential determinant of tidepool community structure and suggests that nutrient excretion by filter-feeding invertebrates may influence the structure and dynamics of rocky intertidal communities.

## 12

### PHYLOGENY OF THE EUGLENOID LORICATE GENERA *TRACHELOMONAS* AND *STROMBOMONAS* BASED ON MORPHOLOGICAL AND MOLECULAR DATA

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Since the separation of the *Trachelomonas* subgroup "Saccatae" into a new genus, *Strombomonas* Deflandre (1930), there has been some question as to its validity. Deflandre's separation was based on morphological characteristics such as the shape of the lorica, lack of a distinctive collar, possession of a tailpiece, lack of ornamentation, and ability to aggregate particles on the lorica. Recent molecular analyses indicated that the loricate taxa were monophyletic, but few species have been sampled. The LSU rDNA from eleven *Strombomonas* and thirty-eight *Trachelomonas* species was sequenced to evaluate the monophyly of the two genera. Bayesian and maximum-likelihood analyses found one monophyletic clade for each genus. The *Trachelomonas* clade was weakly supported, but had five strongly supported subclades. Morphological characters, such as lorica development and pellicle strip reduction, also supported separation of the genera. Lorica development in *Strombomonas* occurred from the anterior of the cell to the posterior, forming a shroud over the protoplast whereas in *Trachelomonas*, a layer of mucilage was excreted over the entire protoplast, followed by creation of the collar at the anterior end. Taxa from both genera

underwent exponential strip reduction at the anterior and posterior poles. In *Strombomonas*, only one reduction was visible in the anterior pole, while in most *Trachelomonas* species, two reductions were visible. Likewise, *Strombomonas* species possessed two whorls of strip reduction in their posterior end compared to a single whorl of strip reduction in *Trachelomonas* species. The combined morphological and molecular data support the retention of *Trachelomonas* and *Strombomonas* as separate genera.

## 13

### VIRAL CONTROL OF PHYTOPLANKTON

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Viral control of phytoplankton Virus ecology is a young rapidly developing field that is changing our understanding of the functioning of marine ecosystems. Viruses are known to be ubiquitous, numerically dominant members of the aquatic microbial communities, infecting a wide spectrum of hosts, including planktonic microalgae. They are typically host-specific agents of mortality and thus likely influence the dynamics and succession of phytoplankton. As well, viral infection can have major biogeochemical implications through cell lysis and the resulting transformation of released POM into DOM. Bloom forming algal species with their high cell abundance may allow a rapid propagation of lytic viruses, resulting in a direct and significant controlling impact of viruses on bloom dynamics. Besides the concentration of host and virus, other potential factors influencing the quantitative importance of viral control of phytoplankton are resistance of host strains to virus infection (host range), variations in morphotypes of the phytoplankton species, the type of limiting nutrient, and variables affecting the fate of viruses (UV, grazing, adsorption to aggregates). The current presentation will provide examples of viral infected phytoplankton and will address some of the processes involved in viral mediated mortality of microalgae.

## 14

### PHYLOGENY OF CHLAMYDOMONADS ISOLATED FROM SALINE HABITATS: INFERENCES FROM 18S and 26S rDNA DATA

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Although the vast majority of chlamydomonadalean organisms are found in freshwater habitats, several

prominent lineages (e.g., *Dunaliella*, *Brachiomonas* and some species of *Chlamydomonas*) are found in brackish, marine or brine environments. Unknown chlamydomonads from Salt Plains National Wildlife Refuge (SPMO; OK USA) and from the Culture Collection of Marine Phytoplankton (CCMP; Bigelow Labs, ME USA) were included in a broad phylogenetic analysis using both 18S and 26S rDNA data. Results indicate that several halotolerant/halophilic lineages can be identified among the Chlamydomonadales. One lineage, which includes *Chlamydomonas parkeae*, *Chl. hedleyi* (symbiont of foraminifer) and five CCMP isolates, is allied in the Tetracystis clade with *Chl. moewusii* and *Chl. noctigama*. A second group, which includes *Chl. reginae*, *Chl. uva-maris* and CCMP 233, is allied with *Chl. monadina*. *Brachiomonas*, which is sister to *Haematococcus lacustris*, has no close halotolerant/halophilic allies. The three SPMO isolates plus CCMP 220 and CCMP 1641 are resolved as close allies of various *Dunaliella* taxa. Two of the SPMO isolates are distinctive from a typical *Dunaliella* cell in that they regularly form non-flagellated cellular aggregations in normal saline media. In summary, these observations collectively suggest that the ability to live in a saline habitat has arisen, independently, in at least four chlamydomonadalean lineages. In addition, substantial taxonomic revision is required to accommodate virtually all of the unidentified isolates included in this investigation. Supported by NSF DEB 9726588, DEB 0129030, MCB 0132083 and MCB 0132097.

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### PHYLOGENY OF THE CHLOROPHYTA: INFERENCES FROM 18S and 26S rDNA

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Recent studies of the Chlorophyceae using 18S and 26S rDNA data in meta-analysis have demonstrated the power of combining these two sets of rDNA data. Furthermore, the 26S rDNA data complement the more conserved 18S gene for many chlorophycean lineages. Consequently, this data approach was pursued in an expanded taxon-sampling scheme for the Chlorophyta, with special reference to the classes Chlorophyceae and Trebouxiophyceae. Results of these new phylogenetic analyses identify *Microspora* sp. (UTEX LB 472) and *Radiofilum transversale* (UTEX LB 1252) as sister taxa which, in turn, form a basal clade in the *Cylindrocapsa* alliance (*Treubaria*, *Trochiscia*, *Elakathrix*). The relative position of the "Cylindrocapsa" clade within the Chlorophyceae remains uncertain. The enhanced taxon-sampling has not resolved the relative positions of the Oedogoniales, Chaetophorales or Chaetopeltidales. Furthermore, the Sphaeropleaceae are supported as

members of the Sphaeropleales in only some analyses, raising concerns about the status of the order. Although based on a limited set of taxa (currently <10), a combined data approach reveals support for a monophyletic Trebouxiophyceae that includes the distinctive organisms, *Geminella* and *Eremosphaera*. The goal of a well-resolved phylogeny for the Chlorophyta remains just that, a goal. Achieving that goal obviously will require additional taxon sampling in the Prasinophyceae and Ulvophyceae, as well as, the Trebouxiophyceae. Moreover, it is clear that other genes (e.g., cp-atpB, cp-rbcL, cp-16S, mt-nad5) will be needed to help address problems of resolution based on the rDNA data alone. Supported by NSF DEB 9726588 and DEB 0129030.

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### COMPARISONS OF MACROPHYTE COVER AND COMMUNITY PRIMARY PRODUCTIVITY ON TWO SOUTHERN CALIFORNIA SHORES

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Light-saturated net photosynthetic rates and cover of rocky intertidal macrophytes were determined between January and March 2003 at two southern California sites characterized by different macrophyte standing stocks. Overall macrophyte cover at Little Corona del Mar was low (75.4%) and was dominated by articulated corallines, and small, turf-forming crustose algae that provide little habitat structure. Macrophyte cover was higher at Dana Point (99.4%), where larger, frondose seaweeds were more abundant (34% vs < 5% cover). Our light-saturated photosynthetic rates for Little Corona del Mar and Dana Point macrophytes were similar to values for the same species obtained during the 1970s and 1980s. Highest photosynthetic rates were obtained for thinner, sheet-like, and branched, frondose seaweeds, while lowest rates were found for articulated coralline and crustose algae. We estimated the net community productivity of the two sites using photosynthetic rates (calculated as  $\text{mg C m}^{-2} \cdot \text{h}^{-1}$ ) and percent cover data for the most abundant populations. We also compared our community productivity estimates for Little Corona del Mar and Dana Point with values for the same sites calculated using macrophyte cover values obtained during the mid-1970s. Re-sampling studies of these and other regional sites reveal that lower-producing, crustose and coralline algae have become increasingly abundant while the cover of higher-producing, frondose algae has declined on many southern California shores. Our studies at Little Corona del Mar and Dana Point, indicate that changing macrophyte abundances can have significant effects on the primary productivity of rocky intertidal communities.

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**RESTORATION TECHNIQUES FOR *NEREOCYSTIS LUETKEANA* (MERTENS) POSTELS ET RUPRECHT (BULL KELP)**

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The establishment and growth of *Nereocystis luetkeana* communities are limited by many environmental and biotic factors. Temperature, light quantity and quality, turbidity, salinity, wave motion, and competition for space and light all determine where populations of this annual kelp will establish and persist from season to season. Like much of the nearshore marine vegetation in Puget Sound and the San Juan Archipelago, *Nereocystis* is negatively impacted by anthropogenic disturbances, such as shoreline development and sediment loading from terrestrial erosion. These events can alter or eliminate optimal habitat and conditions that support adult populations, as well as interfere with the development of the microscopic life stages. Various techniques to establish a *Nereocystis* bed in the northern waters of Washington, USA are examined and will serve as a model for future restoration efforts. Techniques being investigated are as follows: 1) out-planting *Nereocystis* during different seasons; 2) out-planting at different stages of maturity, i.e., zoospore and microscopic sporophyte (0.5–1.0 mm blade length) from cultures; 3) out-planting directly on natural substratum versus at elevated positions; 4) transplanting juveniles (10 cm stipe length); and, 5) seeding natural substratum with out-planted sori (sporophylls), bypassing the lab culturing phase. Results of this study will help define the optimal restoration procedure, including environmental parameters, for *Nereocystis* in order to aid coastal managers in the implementation of nearshore restoration projects. Preliminary results will be presented.

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**MORPHOLOGY, 16S rRNA GENE SEQUENCES, AND INTERNALLY TRANSCRIBED (ITS) REGIONS: TOWARDS A MEANINGFUL SYSTEMATIC FRAMEWORK FOR THE CYANOBACTERIA**

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Cyanobacterial systematics is currently in a state replete with confusion. Many systematic frameworks have been proposed to varying degrees of success. Traditional designated clades (e.g., Chroococcales and Oscillatoriales) have been shown to be polyphyletic, and do not truly reflect evolutionary histories.

Recently, however, research has identified some phylogenetically informative characters at the familial level based on morphological characters. The 16S rRNA gene sequence has been advocated as the gold standard for systematic assignment of members of this clade, although the utility of this gene at assigning higher level taxonomy and species level designations remains questionable. We propose a three pronged approach to cyanobacterial systematics. First, the use of thylakoid arrangement and type of cell division is useful at the familial level. Next, 16S rRNA gene sequence data can be used to assign generic placement and together with morphology is sufficient for recognition of separate species. Third, the secondary folding structure of the 16S-23S rRNA internally transcribed spacer region provides an excellent tool for species level assignments by providing recognizable autapomorphies. We have employed these characters in the analysis of 25 members of the Oscillatoriales, and obtained an excellent degree of resolution and stability in their systematic placement. Taken together, we feel this combined approach can lead to an accurate assessment of cyanobacterial phylogeny and taxonomy in the context of the autapomorphic phylogenetic species concept.

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**PERIPHYTIC ALGAE AS BIOINDICATORS OF NITROGEN INPUTS IN LAKES**

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Increased nitrogen levels has been shown to be a problem in Southern New Jersey lakes, with anthropogenic loadings being the most serious concern (Aber, 1992). It has been suggested that biomass, diversity, and community structure of periphytic algae are good biotic indicators for monitoring water quality and nutrient enrichment in fresh water lakes. (Biggs, 1989). Since, for algae and many other aquatic organisms, nitrogen is one of the most important factors for growth, a good correlation it is expected between nitrogen loading and algal growth. In this bench-scale microcosm study the periphytic community was analyzed using chlorophyll a, dry-ash weight biomass and cell counts, as well as the diversity and community structure for a six-month period, in three tanks with different nitrogen levels (control = non-detectable; low and high). Physical (T/DO/pH) and chemical (nutrients) parameters were measured monthly. Biological parameters were compared with the different nitrogen loading using correlation analysis to show whether nitrogen is a factor in the over-enrichment of New Jersey lakes. To compare the bench-scale microcosm study with the natural settings periphytic algae from two Southern New Jersey lakes, Oswego Lake (no nitrogen) and Oakford Lake (high

nitrogen), were also collected during fall of 2002 to summer of 2003.

## 20

### CHLOROPLAST SIGNAL TRANSDUCTION IN *HETEROSIGMA* AKASHIWO

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Maintenance of homeostasis requires that an organism perceive selected physical and chemical signals and convert this information via signal transduction pathways into an appropriate metabolic response. The *Heterosigma* chloroplast genome encodes the regulatory protein of a His/Asp signal transduction system. This two-component system, which is also found in prokaryotic cells, allows the organism to selectively modify chloroplast gene transcription in response to environmental cues. Using artificially created 50 base oligomers in a TRG1-DNA binding assay, it was determined that this regulatory protein recognized the 6-base consensus sequence TTAGGT. Analysis of 27 sequenced *Heterosigma* chloroplast genes revealed that this six-base motif was found almost exclusively in genes associated with photosystem II, Calvin cycle and energy generation functions with high statistical probability- suggesting that TRG1 may be a global regulator of this gene set. Molecular modeling has shown that the *Heterosigma* TRG1 protein with the sigma 70 subunit of eubacterial-like RNA polymerase which we know from sequencing data to be chloroplast localized in *Heterosigma*. Antibodies generated against TRG1 showed no change in the concentration of this protein when vegetative *Heterosigma* cells were maintained on a 12 h light:12 h dark photoperiod or during resting cell biogenesis. This observation suggests that TRG1, like its bacterial counterparts, is regulated by phosphorylation. The presence of a His/Asp two-component signal transduction pair in the chloroplast genome is taxon-specific.

## 21

### DIFFERENTIAL SCREENING AND SUPPRESSION SUBTRACTIVE HYBRIDIZATION IDENTIFIES GENES DIFFERENTIALLY EXPRESSED IN MALE AND FEMALE PLANTS OF *AGLAOTHAMNION OOSUMIENSE* (RHODOPHYTA)

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Sex determination and development of reproductive structures in *Aglaothamnion oosumiense* were examined by cytogenetic and molecular studies. Chromosome studies of male and female reproductive cells indicated that the sexuality of this species might be determined by a sex chromosome. Chromosome counts in female and male gametophytes were 37 and 36, respectively. The sex ratio of the gametophytes was 1:1. Also male-derived bisexual plants were observed. They were different in morphology, position of carpogonial branches and chromosome number. Some male plants also developed parasporangia. Chromosome number of the parasporangial germlings was the same as in the male plants. A novel method combining element of suppression subtractive hybridization (SSH) with high throughput differential screening permitted an efficient and rapid cloning of rarely transcribed differentially expressed genes. Potential of the method is demonstrated by the isolation of 212 subtractive clones that were differentially expressed in male and female plants. About 18 subtractive clones were confirmed by reverse northern blotting. Two genes, AOMS-1 and AOMS-2, which showed male specific expression were analyzed. These genes seem to be involved in differentiation of male reproductive structures.

## 22

### NUTRIENT USE EFFICIENCY AND COASTAL PRODUCTIVITY: COMPARATIVE PERSPECTIVES ON ECOSYSTEM STRUCTURE AND MECHANISMS OF CONTROL

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Net primary production in marine ecosystems ultimately reflects the inputs of nutrients and the efficiency with which nutrients are acquired and used by phytoplankton in growth. In contrast to our understanding of the linkages between nutrient loading and production, the influence of nutrient use efficiency (NUE) on cross-system variations in coastal productivity remains unclear. Nutrient use efficiency at the ecosystem scale is the product of the per capita efficiency of nutrient use in phytoplankton growth and the efficiency with which phytoplankton communities are able to assimilate limiting nutrient(s). We measured the relative dominance of ecosystem N pools by phytoplankton biomass as an index of NUE across 56 inner-shelf sites. These sites were distributed across a strong geographic range of upwelling intensity and productivity along the coasts of Oregon, California and New Zealand. We also compiled an extensive dataset of published NUE values in coastal and oceanic sites in



order to assess cross-system patterns and differences in NUE. Our results indicate that exceptional rates of productivity in inner-shelf upwelling systems arise as a consequence of near dominance of ecosystem N pools by phytoplankton biomass. Elevated rates of NUE nevertheless appear to be a transient phenomenon in marine systems. Cross-shelf transects across upwelling fronts off the Oregon coast reveal a temporal pattern of intense phytoplankton blooms and decline that reflects the eventual dominance of ecosystems N pools by detrital and dissolved organic N pools. Our findings suggest that NUE may play a central role in governing the productivity of marine ecosystems.

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**PHYLOGENETIC POSITION OF *ALARIA FISTULOSA* (LAMINARIALES, PHAEOPHYCEAE) BASED ON NUCLEAR ITS AND PLASTID CODING GENES**

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*Alaria* is a common kelp genus generally found in the lower intertidal and shallow subtidal regions of rocky shores in the cold waters of the northern Hemisphere. About 16 species are currently recognized worldwide and, of these, *A. fistulosa* is distinguished by having hollow midrib and large blades with 10–30 m in length and 30–90 cm in width. It is the only canopy-producing kelp in the north-west Pacific, where it is restricted to the waters of north Hokkaido, Kamchatka, Aleutian Islands, and Alaska. In order to know the phylogenetic position of *A. fistulosa*, sequences of nr DNA ITS and plastid *rbcL* including spacer and *psaA* regions were determined in *A. fistulosa* and compared with homologous positions of newly sequenced putative relatives and with published sequences of other kelp species. Combined data of ITS and Rubisco spacer show that *A. fistulosa* was more related to the clade of *Lessoniopsis* and *Pterygophora* than to the clade of other species of *Alaria*, which is supported by the *rbcL* and *psaA* sequence data. The topologies from nuclear and plastid DNA sequences lead to phylogenetic independence of *A. fistulosa*, which is clearly different from the genus *Alaria*.

24

**DIVERSITY OF MACROALGAL EPIPHYTES ON *THALASSIA* AND *SYRINGODIUM* IN TAMPA BAY, FLORIDA**

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Blooms of seagrass epiphytes have potentially important economic and ecological consequences in Tampa Bay, one of the Gulf of Mexico's largest estuaries. As part of a Tampa Bay Study to monitor the impact of environmental stresses, we are characterizing epiphyte diversity for efficient management of affected resources for assessment of ecosystem health. We have collected green, brown and red algal epiphytes on *Thalassia testudinum* and *Syringodium filiforme* from fixed monitoring sites at Bishop Harbor, Cockroach Bay, Feather Sound, and from dense and sparse *Thalassia testudinum* and *Syringodium filiforme* seagrass bed sites at Mariposa Key and north of Port Manatee. A main goal of this ongoing study is the determination of indicator species for both healthy and stressed seagrass bed environments.

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**PROPOSAL OF A NEW RED ALGAL GENUS IN THE CERAMIEAE (CERAMIACEAE, CERAMIALES)**

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A new genus is proposed to accommodate four species belonging in the *Ceramium flaccidum* complex based on comparative vegetative morphology and *rbcL* sequence analysis: *C. flaccidum* (Harvey ex Kuetzing) Ardisson (1871), *C. gracillimum*, *C. Agardh* (1824), *C. fimbriatum* S. & G. (1924) and *C. rectocorticum* Dawson 1950. Comparative *rbcL* sequence analysis indicates that this complex forms a distinct monophyletic clade separate from other *Ceramium* species. Although *C. flaccidum* has been placed in *Hormoseras* Kuetzing 1841 (as *H. flaccidum* Harvey ex Kuetzing 1862), the type species of *Hormoceras* is *Ceramium diaphanum*. The new genus is characterized by alternate branching, incomplete cortication, production of three cortical filament initials of which the

basipetal initial divides horizontally, and unicellular rhizoids terminating in pad-like structures produced from periaxial cells. Cortical filament development is discussed for related genera in the tribe Ceramieae (*Campylaeophora*, *Carpoblepharis*, *Centroceras*, *Ceramium*, *Corallophila*, *Herpochondria*, *Microcladia*, *Reinboldiella*). *RbcL*-based phylogenies indicate that the new genus is sister to *Centroceras*, a genus possessing three cortical filament initials. The phylogenetic relationships within the tribe may correlate with cortication patterns that characterize each genus

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**DEVELOPMENT OF THE CULTIVATION OF LAMINARIA SACCHARINA AS THE EXTRACTIVE INORGANIC COMPONENT OF AN INTEGRATED AQUACULTURE SYSTEM AND MONITORING OF THERAPEUTANTS AND PHYCOTOXINS**

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The development of sustainable integrated aquaculture systems requires combining fed aquaculture (finfish) with extractive inorganic aquaculture (seaweed) and extractive organic aquaculture (shellfish). With the support of AquaNet, the Network of Centers of Excellence in Aquaculture in Canada, we are developing such a system at an industrial pilot scale by co-cultivating salmon (*Salmo salar*), kelp (*Laminaria saccharina*) and blue mussel (*Mytilus edulis*) at aquaculture sites in the Bay of Fundy, Canada. This presentation will focus on the development of the extractive inorganic component. The entire cycle of rearing *Laminaria saccharina* has been completed and improved, both in the laboratory and at the integrated sites: release in the laboratory of spores from mature macroscopic sporophytes, seeding of ropes, germination of microscopic gametophytes, sexual maturation of male and female gametophytes, development of zygotes into juvenile sporophytes, which are then transplanted to the sites for rapid grow-out.

Another aspect of the project, food safety monitoring of chemical therapeutants and phycotoxins in mussel and kelp cultured in proximity to salmon, will also be described. The productivity, nutrient absorption capacity, and role of the seaweed component are being analyzed so that its appropriate scale to the other components can be defined in order to develop responsible aquaculture practices in which metabolic/physiological processes of the different co-cultured organisms counter-balance each other within acceptable operational limits. Adopting polytrophic strategies will be key to the aquaculture industry to develop its environmentally and economically-balanced diversification and increase its social acceptability within a broader coastal management framework.

## 27

**SPATIOTEMPORAL DISTRIBUTION OF ABUNDANCE AND DENSITY OF CALCAREOUS GREEN MACROALGAE ALONG THE FLORIDA KEYS: A SYNCHRONY STUDY**

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Discerning patterns of spatiotemporal variation in wide areas is a basic step in understanding changes on coastal communities. Spatial autocorrelation or spatial synchrony (i.e. how populations covary in abundance over time) provides helpful information from population dynamics that allow us to detect common trends of variation at different spatial scales. In this study we describe the spatial and temporal variation of five calcareous green macroalgae, and analyze the synchrony of abundance and density of the five genera separately and as a functional-group along the Florida Keys. For this study we are using data from thirty sites that have been quarterly monitored since 1996. Abundance and density of seagrass and macroalgae have been recorded using a Braun-Blanquet methodology for the Seagrass monitoring program. We found that each genus has a distinct spatiotemporal distribution, different to the group distribution. We evaluated synchronous changes in abundance and density over time at different space scales; we found that synchrony was high between patches and lower between distinct geographical areas; and each genus showed its own synchrony pattern which was obscured when analyzed as a group. We compared the results from functional-group to individual genus analyzing the validity of the use of functional-groups for monitoring programs. We discuss the role of synchrony in the selection of genera for a functional-group.

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**LIPID COMPOSITION OF MEMBERS OF THE ALGAL CLASS CHLORARACHNIOPHYCEAE**

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The algal class Chlorarachniophyceae is comprised of a small group of unicellular eukaryotic algae that are often characterized by an unusual amoeboid morphology. This morphology is hypothesized to be the result of a secondary endosymbiosis in which a green alga was engulfed as prey by a nonphotosynthetic amoeba or amoebaflagellate. Whereas much is known about the phylogenetic relationships of individual chlorarachniophytes to one another, and to possible ancestral host organisms in the genera *Cercomonas* and *Heteromita*, little is known about their physiology, particularly that of their lipids. In an initial effort to characterize the lipids of this algal class, seven organisms were examined for their fatty acid and sterol composition. These included *Bigelowiella natans*, *Chlorarachnion globosum*, *Chlorarachnion reptans*, *Gymnochlora stellata*, *Lotharella amoebiformis*, *Lotharella globosa*, and *Lotharella sp.* Fatty acids associated with chloroplast-associated glycolipids, cytoplasmic membrane-associated phospholipids, and storage triglycerides were characterized. Glycolipid fatty acids were found to be of limited composition, containing principally eicosapentaenoic acid [20:5(n-3)] and hexadecanoic acid (16:0), which ranged in relative percentage from 67–90% and 10–29%, respectively, in these seven organisms. Triglyceride-associated fatty acids were found to be similar. Phospholipid fatty acid composition was more variable. The principal phospholipid fatty acids, 16:0 (25–32%) and a compound tentatively identified as docosapentaenoic acid [22:5(n-3)] (26–35%), were found along with a number of C18 and C20 fatty acids. All organisms contained two sterols as free sterols. These were tentatively identified as 24-ethylcholesta-5,22E-dien-3b-ol (stigmasterol; 70–95%) and 24-methylcholesta-5,22E-dien-3b-ol (brassicasterol; 5–30%).

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**PIN THE PART ON THE DINOFLAGELLATE: A HANDS ON LEARNING ACTIVITY**

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Marine science education often uses animals with high student appeal, such as manatees, sea turtles,

whales and dolphins, to introduce science concepts. The challenge in teaching microalgae concepts is to make the activity as equally appealing and engaging to students. The activity created used an enlarged diagram of a marine dinoflagellate, *Karenia brevis*, to introduce anatomy and function concepts. The activity was simple in design requiring only a large color diagram of the organism, laminated for durability and hook and loop fastener for placement of parts in the appropriate area. Because of its simplicity in design, the activity is inexpensive can be easily reproduced. Key words were also created and laminated to reinforce vocabulary. This activity was found to reach a wide age range from elementary school students to high school. The facilitator could adjust the prompts to assist the students in the proper labeling based on age. At the elementary level, basic anatomy such as nucleus and flagella could be introduced. At the middle school level, labeling of the chloroplasts could lead the discussion to photosynthesis and the food web, and at the high school level the discussion could be focused toward primary productivity and harmful algal blooms. The concepts taught in this activity easily address many key points in the National Science Education standards such as structure and function of living systems and diversity and adaptation of organisms. This activity could easily be adapted to numerous algal organisms in both the marine and freshwater environments.

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**SEASONAL CONTROL OF PHYTOPLANKTON BIOMASS AND PRODUCTIVITY IN COASTAL BRITISH COLUMBIA LAKES AND RESERVOIRS**

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Factors controlling algal abundance and carbon fixation form a cornerstone of aquatic ecology. Central among these are light, nutrients, and grazers. We measured <sup>14</sup>C fixation over one year in six coastal BC lakes that differed in trophic status and grazer community structure. The lakes in our study were never covered with ice, so mixing due to wind energy was more similar to summer months and light levels were higher during winter than comparable ice-covered lakes. Our study, therefore, offered a unique opportunity to examine how seasonal changes in light and temperature affects the functioning of these lakes. While many of our study lakes had higher chlorophyll concentrations during winter months, only the lake with a community dominated by small grazers maintained moderate nutrient deficiency throughout the year and increased <sup>14</sup>C-fixation during the winter (Jan–Feb).

## 31

**EFFECTS OF LIGHT AND AMMONIUM ON GROWTH, AMMONIUM UPTAKE AND PIGMENTATION OF FOUR PORPHYRA (BANGIALES, RHODOPHYTA) SPECIES NATIVE TO THE NORTHWESTERN ATLANTIC**

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The marketability and physiological characteristics of *Porphyra* (*nori*) make it a good candidate for integrated aquaculture. Four species were grown in separate three-week greenhouse trials via tumble culture in small tanks (38L). *Porphyra umbilicalis*, *P. linearis*, *P. leucosticta* and *P. amplissima* were grown at 15, 8, 15 and 12 C, respectively. Plant growth, ammonium uptake, and pigment content were evaluated at three light levels (4%, 13%, and 58% of ambient sunlight), and two ammonium concentrations (25 and 250  $\mu$ M). The growth of *Porphyra umbilicalis* was higher at 250  $\mu$ M than at 25  $\mu$ M ammonium ( $P < 0.002$ ), while growth of the other three species were not affected by nitrogen concentration ( $P > 0.05$ ). With the exception of *P. leucosticta*, plant growth was greatest in high light (58% of ambient). Overall, average growth rates ranged from 31.8 ( $\pm 2.8$ )%  $\cdot$  day<sup>-1</sup> for *P. amplissima* to 8.8 ( $\pm 1.2$ )%  $\cdot$  day<sup>-1</sup> for *P. leucosticta*. Ammonium uptake rates were consistently higher in 250 than 25  $\mu$ M ammonium ( $P > 0.05$ ), except for *P. amplissima* ( $P > 0.05$ ). Uptake by *P. umbilicalis* was greatest at high light ( $P < 0.005$ ), but light did not affect uptake rates in the other three species ( $P > 0.05$ ). The phycoerythrin content of *P. umbilicalis* and *P. leucosticta* was greater at 250  $\mu$ M than 25  $\mu$ M ammonium, while it was not affected by nitrogen level in *P. linearis* and *P. amplissima*. The phycoerythrin content of all species decreased from low to high light ( $P < 0.05$ ); however, light affected the net phycoerythrin production only in *P. leucosticta*.

## 32

**PHYTOPIA: AN EDUCATIONAL CD-ROM TO EXPLORE THE BOTTOM OF THE FOOD WEB**

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“Phytopia: Discovery of the Marine Ecosystem” is an educational CD-ROM that features a wealth of new images, animations, microscope- and satellite-derived data sets, and multimedia discovery tools. It has received support from NASA and the National Science Foundation and can be ordered on-line through <http://www.bigelow.org/phytopia>. A pre-release version of the “Phytopia” was tested by students, educators, and scientists at 34 institutions in Britain, Germany, Russia, and throughout the U.S. This product is designed for use in undergraduate classes; however, our testing has shown that it provides an exciting and interactive learning experience appropriate for Grades 7 and higher. The virtual microscope tool allows the user to view prepared assemblages of plankton at various magnifications, under different epifluorescence conditions, or by scanning electron microscopy. For several species, the user can view movies showing the organism’s motility. Some species are featured as three-dimensional models that can be viewed from any perspective. “Phytopia” also helps users connect ocean primary productivity patterns with environmental factors in several geographic areas. Innovative tools allow investigation of co-registered temperature, wind, current, nutrient, and ocean color data. This tool is aligned with research efforts to better understand plankton ecology using remotely sensed data.

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**BORON IN DIATOMS**

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Lewin, J. C. (1965) showed that boron is required for the growth of *Cylindrotheca fusiformis*, and, presumably, other diatoms too. It may be involved in cellular metabolism, as in higher plants, or incorporated by accident or design into the siliceous skeletons (frustules), since B<sub>2</sub>O<sub>3</sub> has been proven to replace SiO<sub>2</sub> (e.g. in siliceous rocks). Thus the frustules may contain or bear at their surface a proportion of borosilica, which might reduce their solubility in water. (Incidentally, boron has also been implicated in human bone formation and stability (Newnham, 1994). Deyhle (2002), who determined the content of B in calcareous skeletons of marine foraminifera and compared the isotopic ratios of 11<sub>B</sub>/10<sub>B</sub> with that in the environmental sea water, found the B content to be generally less than 10 ppm. We sought comparable values for diatom frustules, washed and bleached to remove salts and organic matter, if possible without leaching away any of the B that could be present. We studied fossil diatomite from Lompoc, California; diatomaceous ooze from Myvatn, Iceland; natural plankton diatoms harvested from the sea; and pure cultures of marine and freshwater diatoms grown in the laboratory. By contrast with foraminiferan calcite,

our data indicated that diatom frustules contain up to 1,600 ppm of boron, with a more marked enrichment of the lighter isotope. Rough calculations indicate that the  $B_2O_3$ , even if concentrated at the frustule surface, might be enough to reduce the solubility of the frustules. Our B-isotope enrichment data range between +3.9 to -0.2‰, indicating that diatoms preferentially incorporate the light  $^{10}B$ . Such values can provide indications to physico-chemical conditions under which the skeletons were deposited (cf. Lewin, E. et al. 2000) Data will be presented.

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#### **PIECES OF A PUZZLE: BIOGEOGRAPHY OF SOUTHEAST FARALLON ISLAND, CALIFORNIA**

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Rocky intertidal communities in cold waters on open shores tend to have a stable, predictable makeup worldwide. The structure of the environment, the morphology and life history of species, the economics of species behavior and the dynamics of population changes all contribute to the distribution of species in a given habitat. South East Farallon Island, in the Pacific Ocean off the northern California coast, hosts an intertidal community typical in many ways of other rocky intertidal communities. However, two orders of marine algae one might expect to be there, the Fucales and Laminariales, are unexpectedly uncommon on this island. In this paper some of the possible environmental, morphological and life history factors contributing to and restricting the distribution of marine algae in the intertidal zone on South East Farallon Island are considered.

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#### **DIVERSITY AND DISTRIBUTION OF FUSIFORM MICROCHLOROPHYTE ALGAE FROM ITASCA STATE PARK, MINNESOTA, AND ARROWWOOD NATIONAL WILDLIFE REFUGE, NORTH DAKOTA**

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Several types of fusiform microchlorophyte algae (*Monoraphidium*, *Ankistrodesmus*, and related genera) have been characterized from lakes and ponds of Itasca State Park, Minnesota, and Arrowwood National Wildlife Refuge, North Dakota. PCR-RFLP analysis of 18s ribosomal RNA genes was used to categorize 29 different isolates from Itasca State Park

into 9 different types, and 15 isolates from the Arrowwood National Wildlife Refuge samples into 8 different types. 18S ribosomal DNA sequences were determined for at least one isolate from each type for phylogenetic analysis. All of these sequences were different than any sequence published in GenBank. None of the types were found in both Itasca State Park and Arrowwood National Wildlife Refuge. These results suggest that fusiform green algae are highly diverse, with little species overlap in different habitats.

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#### **NUTRIENTS AND HARMFUL ALGAL BLOOMS: GENERAL ISSUES AND EXAMPLES FROM THE NORTHERN GULF OF MEXICO**

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Nutrient inputs have increased worldwide in both marine and freshwater ecosystems, stimulating algal growth. The resulting increased eutrophication can be manifested in different ecosystems as hypoxia/anoxia, loss of submerged aquatic vegetation, or Harmful Algal Blooms (HABs). HABs can significantly impact human and ecosystem health. It has been hypothesized that HABs are increasing worldwide, in part because of increasing nutrient inputs, but establishing a direct cause and effect link has been difficult for a variety of reasons. Two examples from the Louisiana coastal zone illustrate some of the difficulties and successes: annual *Pseudo-nitzschia* spp. blooms on the shelf in the extended plume of the Mississippi River and sporadic, toxic cyanobacterial blooms in low salinity estuaries. The challenge is to determine if a HAB is caused by increased nutrient inputs and which nutrient(s) are the main culprit(s), to identify the source(s) of the increased nutrients, and to develop effective management strategies. The latter is often a social/political problem that must be well-informed by the science.

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#### **PRODUCTION, SURVIVAL AND MORPHOLOGY OF KELP (PROTISTA) "SPOROPHYTES" ARISING FROM SELFINGS, APOGAMY AND HYBRIDIZATIONS**

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The production and survival of sporophyte morphologies that arose from selfings, parthenogenesis, male apogamy and reciprocal hybridizations of *Costaria*

*costata* (C.A. Agardh) Saunders, *Laminaria saccharina* (L) Lamouroux, *Alaria marginata* Postels and Ruprecht, *Eisenia arborea* Areschoug, *Lessoniopsis littoralis* (Tilden) Reinke, *Macrocystis integrifolia* Bory, and *Nereocystis leutkeana* (Mertens) Postels and Ruprecht was tracked for 215 hybridization trials for 30 weeks. Each trial consisted of selfings for each species, solo cultures of males and females for each species and reciprocal crossings. At the end of 30 weeks, "sporophyte" survival in the 215 cases was 13% for selfings, 10% for parthenogenesis, 6% for apogamy, and 16% for the reciprocal hybridizations. The morphologies of sporophytes resulting from selfings were typical of the selfed species. Parthenosporophytes and reciprocal hybrids usually had the distinctive morphology of the female parent. Male, apogamously derived "sporophytes" often had reduced morphological features. For example, *A. marginata* male apogamous sporophytes often lacked a midrib. The sporophyte morphologies that arose in the reciprocal crossings are considered to be putative hybrids. Molecular evidence is required to establish their genetic origins.

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### THE SYSTEMATICS OF *OCHROMONAS* (CHRYSOPHYCEAE)

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*Ochromonas sensu lato* is the largest genus in the Chrysophyceae, containing over 100 names. *Ochromonas* species are biflagellate, naked, plastid-bearing single cells, distinguished from loricate, scaled, colonial and colorless genera. Most, if not all, species of *Ochromonas* are mixotrophic, i.e., they photosynthesize but they also engulf bacteria and other small prey. Preliminary evidence from SSU rRNA sequences show that *Ochromonas* is a polyphyletic genus. *Ochromonas tuberculata* is the most distinct from all other *Ochromonas* species. The other *Ochromonas* species (examined thus far) are scattered in three clades. For example, *O. danica* and *O. sphaerocystis* are sister to *Poterioochromonas stipitata* and *P. malhamensis*. Four additional species (identified by light microscopy as *O. elegans* Doflein, *O. globosa* Skuja, *O. ovalis* Doflein, *O. sociabilis* Pringheim) have SSU rRNA sequences identical to *P. malhamensis*. Of these, only *O. sociabilis* has been transferred to *Poterioochromonas*. Thus, at least some species may be synonymous with others. Two clades of marine species are also known, one containing coastal species and the other containing open ocean species. A number of genera (some also polyphyletic) are interspersed amongst the *Ochromonas* species (e.g., *Chrysolepidomonas*, *Chrysonephele*, *Chrysoxys*, *Cyclonexis*, *Dinobryon*, *Epipyxis*, *Uroglena*, *Uroglenopsis*). The goal of this research (just begin-

ning) is to establish a monophyletic *Ochromonas*, probably by assigning some species to other genera (existing or new). One major problem is that the type species, *O. triangulata* Vysotskii, hasn't been observed in over 100 years, and it is unclear which of several clades of *Ochromonas* contains the type. Results will be discussed.

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### PHYLOGENY OF AULACOSEIRA (BACILLARIOPHYTA)

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The phylogeny of 67 populations representing 45 species of *Aulacoseira* is estimated by maximum parsimony methods using a combination of nucleotide sequence data and qualitative and quantitative morphological characteristics of the silica cell wall gathered primarily from original observation by LM and SEM. A new type of character employing continuous quantitative variables describing the ontogenetic-allometric trajectories of cell wall characteristics over the life cycle (size range) of diatoms is introduced. In addition to the 45 *Aulacoseira* species, the phylogeny also incorporates one *Alveolophora* species, and two outgroup species (*Melosira varians* and *Stephanopyxis cf. broschii*). Fifteen species, represented by 24 populations, also contain molecular data from the chloroplast genome (rbcL) as well as the nuclear genome (18S), which were sequenced or downloaded from GenBank. The phylogeny of *Aulacoseira* is composed of five major clades: 1) an *A. crenulata* and *A. italica* clade, which is the most basal, 2) an *A. subarctica* and *A. distans* clade, 3) an *A. granulata* complex clade, 4) an *A. ambigua* clade, and 5) an *A. islandica*, *A. skvorzowii*, *A. baicalensis*, clade that also contains *Alveolophora* and many extinct *Aulacoseira* taxa. Monophyly of *Aulacoseira* is only achieved if *Alveolophora*, originally identified as *Aulacoseira*, is no longer given separate generic status.

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### PHYTOPLANKTON BIOMASS AND COMMUNITY DYNAMICS IN THE LAKE PONTCHARTRAIN ESTUARY OF SOUTHEAST LOUISIANA, USA: EFFECTS OF LIGHT AND NUTRIENTS UPON SEASONAL AND SPATIAL PATTERNS

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A yearlong study (2002–2003) was conducted in the Lake Pontchartrain Estuary (LPE) of Southeast Louisiana in order to examine the temporal and spatial variability of phytoplankton biomass and community dynamics in relation to nutrients, light climate and other abiotic parameters. Variability was assessed through a north-south transect across the estuary and at five of the major tributaries of the LPE at bi-weekly intervals. Phytoplankton pigmentation was analyzed by HPLC and used to calculate biomass (as chlorophyll a) and to determine the relative abundances of phylogenetic groups and taxa (via CHEMTAX). Microscopic analyses of select samples were used to verify the efficacy of the CHEMTAX method in the waters of the LPE. Water-column up-/down-welling irradiance (PAR) and colored dissolved organic matter (CDOM) measurements were used to characterize the light climate. Results indicate that LPE phytoplankton biomass and community composition are: 1) highly variable at the spatial and temporal scales of this study, 2) can significantly impact the light field of the LPE, and 3) are influenced by macro-nutrient ratios.

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#### INCIDENCE OF PARALYTIC SHELLFISH TOXIN IN BIVALVE MOLLUSC TISSUE FROM THE OREGON COAST

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Saxitoxin and domoic acid sequestration by bivalve molluscs occurs periodically along the Oregon coast, presumably as a result of harmful algal blooms (HABs). Since 1958 and more continuously since 1979, the Oregon Shellfish Program (OSP) has assayed toxin levels in these molluscs as part of a monitoring program for paralytic (PSP) and amnesic (ASP) shellfish poisoning. We have created a working data base for all PSP sampling by the OSP between 1958 and 2001 and have examined the data for spatial and temporal trends in the appearance of toxin in shellfish, amount of toxin, and apparent duration of toxic events. In this report, we examine the data from the five stations with the longest record of continuous sampling (1979–2000) for evidence of correlation with El Niño events, upwelling, and/or a pattern of increasing frequency or intensity of toxic events. We also compare the pattern of appearance of toxin at open coast stations with the timing of first appearance of toxin in shellfish at adjacent estuarine stations. This is an important analysis because, in Oregon, shellfish closures due to PSP occur frequently in mussel beds on the open coast and the source of toxin-producing organisms is unknown.

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#### DIVERSITY OF PHYCOERYTHRIN-CONTAINING PICOCYANOBACTERIA THAT SHARE THE SAME SPECTRAL PHENOTYPE

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Phycocerythrin (PE) is an important light-harvesting pigment for many marine picocyanobacteria. There are numerous spectral forms of PE, which differ in their ability to absorb blue and green wavelengths of light, depending on the precise chromophore composition. It remains unclear how the evolutionary history of this family of proteins relates to the diversity of the marine picocyanobacteria. In order to ascertain the level of diversity present in marine picocyanobacterial communities and to determine if specific spectral phenotypes are homologous or convergent, we have begun to examine the genetic diversity found in marine picocyanobacterial strains that share the same or similar spectral signatures for PE fluorescence. The strains we examined are all clonal cultures isolated from a range of environments, including the Arabian Sea and Black Sea. We report results from sequence data obtained using degenerate primers for 16S rRNA, rpoC1, and PE genes; we also examine the utility of taxonomically grouping these organisms by using physiological characteristics such as size and PE signature.

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#### TESTERIA GEN. NOV. (DINOPHYCEAE) FROM MANATEE CAY BELIZE, CENTRAL AMERICA

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A new marine dinoflagellate genus, *Testeria* gen. nov., is reported from coral reef-mangrove pond. A new generic name is proposed for two species: *Testeria novellus* sp. nov. and a new combination for *Testeria steidingerae* (Balech) comb. nov. These two species differ in thecal plate morphology from *Protooperidinium* species based on the following characteristics. Plates of *Testeria novellus* and *T. steidingerae* exhibit: 1) a wide, symmetric, ortho apical plate 1' disconnected from a slender apical horn and in direct contact with the cingulum. 2) apical pore complex absent. 3) anterior intercalary plate (2a) hexa situated dorsally. The thecal plate formula is as follows: 4', 3a, 7'', 4c)3 + t), 5''', 6s, 2'''''. Cells are colorless or pale pink, chloroplasts absent. The similarities and differences of *Testeria* and *Protooperidinium* are discussed with respect to their taxonomical history, species associations and ecology.

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**DIVERSITY OF THE PICOPLANKTON CHORICYSTIS (TREBOUXIOPHYCEAE, CHLOROPHYTA) FROM MINNESOTA AND NORTH DAKOTA LAKES**

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Choricystis (Trebouxiphyceae, Chlorophyta) is considered a very common member of the freshwater picoplankton. We have established a culture collection from Itasca State Park (ISP), Minnesota, and Arrowwood National Wildlife Refuge (ANWR), North Dakota, that includes many Choricystis isolates. We examined 109 Choricystis isolates from ISP and 19 isolates from ANWR using PCR-RFLP of the *rbcL* gene. Twelve types for ISP and 7 types for ANWR were distinguished by this technique. Sequence analysis revealed additional diversity within some of the RFLP types. Moreover, none of the Choricystis isolates from ANWR possessed *rbcL* sequences identical to any isolate from ISP. Phylogenetic analysis of the sequence data revealed at least 2 major lineages. These results indicate that Choricystis is much more diverse than previously thought. This material is based upon work supported by the National Science Foundation under Grant Nos. DEB-0128953, DBI-0070387 and MCB

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**PHYLOGENY AND SYSTEMATICS OF PSEUDODICTYOSPHAERIUM, MYCHONASTES AND RELATED COCCOID GREEN ALGAE (CHLOROPHYCEAE).**

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Species of *Mychonastes*, *Pseudodictyosphaerium*, and *Korshpalmella* all have spherical (or nearly so) coccoid cells without pyrenoids. *Mychonastes* and *Pseudodictyosphaerium* are apparently frequent components of the picoplankton of lakes, although they had not been reported from North America prior to our studies. We have examined several isolates of *Mychonastes*, *Pseudodictyosphaerium*, and *Korshpalmella* from lakes and ponds in Itasca State Park, Minnesota (USA) and Arrowwood National Wildlife Refuge, North Dakota (USA). These isolates comprise 5 different 18S rDNA sequence types, none of which have a sequence identical to any published sequence for *Mychonastes* or *Pseudodictyosphaerium*. Four of these 18S types

produce solitary cells, consistent with *Mychonastes* spp. However, other isolates all have identical 18S rDNA sequences, but are either unicellular, colonial with connecting stalks, or colonial in an amorphous matrix, consistent with *Mychonastes* spp., *Pseudodictyosphaerium* spp. or *Korshpalmella* spp, respectively. For some isolates, the colonial character is plastic, with different morphologies seen with different growth conditions. Ribosomal ITS and *rbcL* sequences also indicate little or no diversity among the isolates with various morphologies. These results suggest that the colonial habit is not a reliable generic character. Phylogenetic analyses of both *rbcL* and 18S rDNA sequences indicate that *Mychonastes* and *Pseudodictyosphaerium* spp. are a distinct lineage within the Chlorophyceae and should be placed in a new family. This material is based upon work supported by the National Science Foundation under Grant Nos. DEB-0128953, DEB-0129030, DBI-0070387 and MCB-0084188.

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**NEAR SHORE SEDIMENT DIATOMS OF THE GREAT LAKES AND THEIR USE AS BIOLOGICAL INDICATORS**

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The Great Lakes Environmental Indicators (GLEI) project, funded through the U.S. EPA, has set goals to identify taxa that will be useful in determining ecosystem integrity in near shore waters of the Great Lakes ecosystem. Through the development of biological indices, rapid, consistent, and inexpensive methods of bioassessment of the Great Lakes can be utilized. This particular study focuses on the use of near shore sediment diatoms. Sediment core collection and water sampling and analysis were completed at nearly 100 stratified randomly selected sites across all five of the Great Lakes. Canonical correspondence analysis (CCA) indicated a gradient of water quality throughout the Great Lakes. Lake Superior was determined to have the lowest concentration of measured nutrients, while Lake Erie and Lake Ontario had the highest. Lake Michigan and Lake Huron shared a similar transitional water quality. Although analyses and indices have been developed thus far, GLEI is in its third year of a 4-year schedule.

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**PHENOLOGY OF PARALEMANEA ANNULATA (LEMANEACEAE, RHODOPHYTA) IN A LOW-ORDER WOODLAND STREAM**

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*Paralemanea annulata* (Kütz) Vis et Sheath, a member of Lemnaceae (Batrachospermales, Rhodophyta), is patchy in its distribution across North America, but seems to be most prevalent in the southern United States. Although the stages in the life history of this alga are well-documented, only scant details are available about the timing of these events and which environmental factors may act as cues. Studies conducted on other members within this family showed correlations between growth and environmental parameters such as daylength, temperature and current velocity. Our study was conducted to investigate whether *P. annulata* was similarly affected by environmental parameters. A stream reach with varying current velocity and light regimes was investigated. Macroscopic gametophytes were present December-July and had spermatangia present in all months except December. The presence of carposporophytes in thalli was observed in May and June. The thalli were heavily colonized by *Audouinella hermannii* March-June after which the gametophytes were not present. This also corresponded with the drying up of the stream and increased water temperature. New growth of gametophytes was observed in December. Gametophyte percent cover showed a significant positive correlation with both current velocity ( $p < 0.001$ ) and light intensity ( $p < 0.001$ ). Other parameters were correlated for certain regions of the stream reach based on light and current velocity regimes.

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#### THE EFFECTS OF ENVIRONMENTAL FACTORS ON THE RESUMPTION OF PHOTOSYNTHETIC ACTIVITY OF CYANOBACTERIAL MATS FOLLOWING REHYDRATION

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Extensive cyanobacterial mats cover the intertidal zone near Guerrero Negro, Baja California Sur. These mats are exposed to extreme desiccation and osmotic stress between tidal flows and rains, and spend most of the time dry and metabolically inactive. Therefore, periods of hydration are extremely important for growth as well as for repair of cellular damage from desiccation and ultraviolet radiation (UVR) accrued when the mat is dry. PAM fluorometry in conjunction with carbon incorporation assays were used to determine the effects of salinity, irradiance and UVR on the recovery of photosynthetic activity in these mats after an extended period of desiccation. The mat used in our study was primarily composed of *Lyngbya* sp. Photosynthetic activity recovery rates (using PAM fluorometry) decreased with increasing salinity. This trend was similar under high and low

light intensities, but rates were significantly lower under low light. Alternatively, the carbon incorporation method showed rates increased faster in salinities of 27 and 55 ppt than in salinities of 0 or 75 ppt. The *Lyngbya* mat also failed to recover photosynthetic potential in the dark. Although these mats recovered faster under high intensity light, the effect of salinity on photosynthesis is more complex. UVR did not affect the recovery of photosynthetic activity, no matter which method was used. This lack of effect is most likely due to the high content of the UVR screening pigment, scytonemin, in the upper layer of the mat.

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#### USE OF BIOMARKERS AND CORE INCUBATIONS TO INVESTIGATE HOW CHANGES IN BENTHIC ECOSYSTEMS INFLUENCE SEDIMENT N FLUXES IN WESTERN AUSTRALIA

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The west coast of Australia is dominated by warm, oligotrophic waters, shallow carbonate-rich sediments and little terrigenous nutrient input. Irradiance and light penetration are generally high so that benthic primary production is substantial and can equal that in the water column. Microphytobenthos has been shown to have a major influence on nutrient cycling in northern hemisphere waters, but little work has been published on this subject describing the Western Australian marine environment. This presentation focuses upon anthropogenic impacts on microphytobenthos and sediment-water column nitrogen cycling. The effects of artificial enclosures (harbours) on microphytobenthic communities and nitrogen cycling were examined over an annual cycle at 2 locations in Cockburn Sound, Western Australia. Core and bulk sediment samples were taken from bare sandy areas in water 6–7 m deep at paired sites inside and outside sea walls. Ex-situ core incubations were used to determine rates of N<sub>2</sub> fixation, denitrification, fluxes of macronutrients and oxygen. Bulk sediment samples were analysed for pigments, fatty acids, sterols and grain size. Significant differences were found in both flux and biomarker data between the 2 areas, inside and outside the harbours and between summer and winter. Biomarker data gave information about the algal, bacterial and faunal composition of the sediments and how it changed across the same temporal and spatial scales as the fluxes. The combination of process studies and biomarker information promises to be a powerful

tool for resolving a range of questions on the magnitude and mechanisms of nitrogen inputs and exports from these shallow ecosystems.

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**CAN THE EFFECTS OF OCEAN WARMING ON TEMPERATE REEF COMMUNITIES BE ACCURATELY PREDICTED? LESSONS FROM A LARGE SCALE, LONG TERM FIELD EXPERIMENT IN CENTRAL CALIFORNIA**

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One of the most commonly predicted effects of global ocean warming on marine communities is a poleward shift in the distribution of species with an associated replacement of cold-water species by warm-water species. Such predictions are imprecise and based largely on broad correlations in uncontrolled studies that examine changes in species composition and abundance relative to seawater temperature. Before-After-Control-Impact (BACI) analyses of the effects of a large thermal discharge shows that an induced 3.4 deg. C rise in seawater temperature over 10 years along 2 km of rocky coastline resulted in significant community-wide changes in 150 species of algae and invertebrates relative to controls. Contrary to predictions from biogeographic models, there was no trend towards warm-water species with southern geographic affinities replacing cold-species with northern affinities. Instead, communities were greatly altered in apparently cascading responses to changes in abundance of several habitat-forming taxa, particularly subtidal kelps (e.g. *Pterygophora californica*) and intertidal foliose red algae (e.g. *Mazzaella flaccida*). Many temperature sensitive algae decreased greatly in abundance, whereas many invertebrate grazers increased. The results indicate that the responses of temperate reef communities to ocean warming can be strongly coupled to direct effects on habitat-forming taxa and indirect effects operating through ecological interactions. Given our understanding of temperate reef ecology and its local variability, the results also suggest that accurate predictions of the effects of global ocean warming will be difficult to make.

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**CHARACTERIZATION OF THE NORTHWESTERN GULF OF MEXICO MACROALGAE**

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The upper Northwestern Gulf basin is characterized by a relatively shallow but well developed continental shelf with an extensive system of mid- and outer shelf hard banks formed on unique tectonically created salt diapirs of varying origin and composition. Collections made by our laboratory from ~ 30 m depth by SCUBA while participating in monitoring cruises to the National Marine Sanctuaries of the East and West Flower Garden Banks (Texas), Stetson Banks (Texas) and Sonnier Banks (Louisiana), and while dredging off Louisiana at ~ 45–90 m depth, reveal a surprisingly different floristic composition among the sites. Several range extensions, new macroalgal records for the Gulf of Mexico, and the establishment of new species for the region indicate that the NW Gulf encompasses complex macroalgal biota. Our ongoing project includes the development of a modern taxonomic, phylogenetic, morphological and genetic database essential to informed management for preservation of biological diversity. It also provides a framework for seasonal algal composition against which long-term trends and anomalies in algal distribution and health of the Gulf of Mexico can be assessed at future dates.

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**BETA-DIMETHYLSULFONIOPROPIONATE (DMSP) AS A GRAZING DETERRENT IN COASTAL PHYTOPLANKTON COMMUNITIES**

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While not much is known about the role of chemical defenses in marine planktonic systems, it has been hypothesized that beta-dimethylsulfoniopropionate (DMSP) may act as a signal for the presence of potentially harmful algal cells. Previous laboratory experiments indicate that DMSP can inhibit feeding in some species of protists, but it is unknown whether this holds true when DMSP is present in coastal communities. Using laboratory and field experiments, the role of dissolved DMSP as a chemical defense in ambient seawater was examined. DMSP (twenty micromolar) added to both laboratory cultures and natural planktonic communities led to a decrease in protist feeding rates; in natural communities, feeding on phytoplankton greater than twenty microns in size was affected more strongly than feeding on smaller cells.

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**A SEAWEED'S PERSPECTIVE ON MARINE RESERVES**

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In response to major changes in coastal ecosystems in recent decades, a number of governmental agencies around the world are establishing marine reserves – areas where removal of animals or plants is prohibited. Although marine reserves are touted as an ecosystem based approach to management of marine resources, the vast majority of attention on reserve design and impact focuses solely on fish. Although a few species of algae are commercially harvested, most are not. As a result, they will receive little direct benefit from protection by reserves aside from habitat protection. From the perspective of a seaweed, the primary impacts of marine reserves will therefore be indirect through species interactions. We examine the rapidly growing theoretical and empirical literature on marine reserves to anticipate the likely responses of seaweeds to exclusion of fishing. The key issues that emerge are: the trophic level of prior fishing and the dispersal scales of seaweeds relative to their competitors and consumers. The latter issue is poorly understood and poses a key challenge to phycologists if we are to effectively incorporate seaweeds into future marine reserve design.

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#### **BENTHIC MARINE ALGAL HERBARIUM OF LONG ISLAND SOUND DIGITAL COLLECTION**

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The University of Connecticut presents the “Benthic Marine Algal Herbarium of Long Island Sound Digital Collection.” The collection is available on the web for teaching and identification purposes. Phase One of this project will be finished later by early next year. Currently, the collection has approximately 60% of all Long Island Sound macroalgae species. The taxonomy is part of a collaboration between the University of Connecticut and the Northeast Algal Society (NEAS). Thus far, the specimens have come from Dr. Charles Yarish’s herbarium and from the Environmental Laboratory at Millstone Power Station on Long Island Sound. The botanical illustrations are from “Illustrated Key to the Seaweeds of New England” by Martine Villalard-Bohnsack. Database features include the ability to create searches and generate sets based on subjects, division, class, order, family, genus, habitat, species, keyword, and location. Data retrieved includes JPG image, cataloged data, map regions, synonyms, and illustrated microscopic, cross section and surface view for some species. The web site is available for use at: <http://www.algae.uconn.edu>

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#### **ICE AGE KELP FORESTS: CLIMATE-DRIVEN CHANGES IN KELP FOREST DISTRIBUTION SINCE THE LAST GLACIAL MAXIMUM**

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Kelp forest distributions are constrained by the availability of rocky substrate within the depth range tolerable for growth and reproduction, which can vary over relatively short geological timescales (millennia) due to interactions between coastal bathymetry and climate-driven changes in eustatic sea level. Using GIS, a digital bathymetric map, sea level curves, and published kelp depth tolerances, I reconstructed changes in the size and distribution of giant kelp (*Macrocystis pyrifera*) forests in the Southern California Bight since the last glacial maximum. Reconstructions predicted that the total area of available kelp forest habitat for the California Channel Islands during the last glacial maximum (18.5 kyr BP; 628 square km) was greater than at present (382 square km) but less than at 16.5 kyr BP (1130 square km). Available kelp forest habitat along the southern California mainland also increased rapidly from 18.5 to 16.5 kyr BP but continued to increase with sea level rise. Differences in the effects of sea level rise on coastal geomorphology between the islands and mainland further constrained the extent of rocky substrate available to kelps. Given biomass and productivity estimates from present-day kelp forests, these reconstructions suggest more productive and spatially extensive island kelp forests near the last glacial maximum than at present, but the opposite pattern for the mainland. These climate-driven changes in kelp forest distribution and productivity likely had important historical impacts on the ecology and evolution of the present-day kelp ecosystem including kelp forest exploitation by early human inhabitants of southern California.

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#### **ALGAEBASE**

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AlgaeBase (<http://www.algaebase.org>) is a web-searchable store of information on those protists generally considered to be algae. Access is free and some 10,000 browser searches on average now take place each month. The database was established in 1996 and at first only included seaweeds. Its main function at this time was as a catalog of the marine algae of Europe for the European Union-funded BioMar and European Register of Marine Species (ERMS) projects,

and for the recently published Atlas and Check-list of the Seaweeds of Britain and Ireland. The data are now being extended to cover all algae. Over 50,000 names, of which about half are presently accepted species names, are now included, together with the names of some 3500 genera, about 3000 common names, approximately 700 pictures, and in excess of 28,000 literature references. URL-based links from a number of other databases including the Species 2000 Annual Check-list, BIOSIS, GenBank, and Codes for Australian Aquatic Biota have been implemented. It is intended to initiate similar connections from new initiatives such as EuroCat and SPICE, and a number of other global biodiversity databases. As part of a further EU-funded project, SeaweedAfrica (<http://www.seaweedafrica.org>), AlgaeBase is being completely rewritten as an SQL database with a browser-enabled interface, enabling access by a panel of taxonomic experts. AlgaeBase hopes thereby to continue to provide high-quality access to community-serviced data in the best traditions of the Internet.

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**SYSTEMATICS OF *HYDROPUNTIA MONTAGNE* (GRACILARIACEAE, GRACILARIALES, RHODOPHYTA) BASED ON COMPARATIVE MORPHOLOGY AND *RBCL* SEQUENCE ANALYSIS**

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The taxonomic validity of the genus *Hydropuntia Montagne* (1843) (including *Polycavernosa*) within the Gracilariaceae (Gracilariales, Rhodophyta) is controversial. Morphological characters that define species of *Hydropuntia* are said to be variable and to overlap with those of *Gracilaria*. Here we present a global phylogenetic study of the family based on a Bayesian analysis of a large *rbcL* DNA sequence dataset indicating that the genus *Hydropuntia* forms a well supported monophyletic clade within the family, and recognize *Hydropuntia* as a genus distinct from *Gracilaria*. We also conducted smaller phylogenetic analyses in which thirty four *Hydropuntia rbcL* sequences resulted in two major clades within the genus, comprising a Caribbean clade and an Indo-Pacific clade. Diagnostic reproductive stages that separate these two clades will be illustrated.

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**DETERMINING THE TOTAL DIETARY FIBER CONTENT IN HAWAIIAN MARINE ALGAE**

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In Hawaii, many species of marine algae are eaten by people, as well as by green sea turtles, reef fish, shore crabs and other herbivorous invertebrates. The Total Dietary Fiber (TDF) content was determined for 30 species (17 Rhodophyta, 4 Phaeophyta, and 9 Chlorophyta) using an enzymatic-gravimetric procedure. Overall, the seaweeds were rich in fiber. TDF values ranged from 58.48% dry wt. in *Ahmfeltiopsis concinna* (J. Agardh) Silva et DeCew to 12.80% dry wt. in *Codium reediae* Silva.

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**UTILITY OF THE PERIPHYTON INDEX OF BIOTIC INTEGRITY (PIBI) AS AN INDICATOR OF ACID MINE DRAINAGE IMPACTS IN SOUTHEASTERN OHIO**

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In the Appalachian region, numerous streams are polluted with acid mine drainage (AMD). These waters are sulfate-rich with elevated amounts of total acidity, low pH, and high levels of dissolved metals. Biotic multimetric indices, such as the Periphyton Index of Biotic Integrity (PIBI) have been employed to determine water quality across a variety of environmental conditions and may prove useful for AMD impacts. This study was initiated (1) to evaluate the PIBI for distinguishing AMD impact in streams and (2) to examine whether PIBI scores are impacted by seasonal differences. Twelve AMD and three reference streams were sampled for periphyton in June, August, and October. Water chemistry was collected at least once during the sampling period. Preliminary results showed that PIBI scores were significantly different ( $p < 0.05$ ) among the seasons. In addition, the seasonal trends in PIBI scores among streams were not consistent. The PIBI scores were correlated with six water chemistry variables in August and with at least one variable indicative of AMD in each season sampled. PCA and UPGMA analyses of water chemistry data grouped the streams into five categories: (1) moderately impacted AMD streams with lower total dissolved solids, sulfate, total aluminum, and alkalinity, and higher sulfate; (2) AMD streams with higher alkalinity and lower total

aluminum; (3) AMD streams with lower alkalinity and higher total aluminum; (4) reference streams and (5) an outlier reference stream affected by nutrients. Relationships between the groups based on water chemistry and the groups derived from the PIBI will be discussed.

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#### **IDENTIFICATION OF DIATOM-LYTIC BACTERIUM SK-02 AND ITS CAPABILITY TO DEGRADE *STEPHANODISCUS HANTZSCHII***

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In order to control harmful algal blooms, many biological approaches have been tried. Specially, there have recently been discussions concerning the roles of bacteria in algal bloom dynamics. Then, algicidal bacteria are expected as an agent considerate for harmful algal blooms control. Development of these organisms as biological control agents involves isolation from environmental samples. With the aim of develop eco-technology controlling water blooms in fresh waters, we isolated the diatom-lysing bacteria from the sediments of Lake Seokchon and Palitang River-Reservoir. A soft agar-overlay technique was used to isolate the diatom lytic bacteria. The SK-02 showed a diatom lytic activity against *Stephanodiscus hantzschii*. Taxonomic identification including 16S rDNA base sequencing, and phylogenetic analysis indicated that the isolate SK-02 had a 99.20% homology in its 16S rDNA base sequence with *Pseudomonas putida*. The nature of these diatom-lying components is still under investigation. These results suggest that the indigenous bacteria isolated from the sediments may have a potential in the application and development of eco-technology controlling harmful water blooms in the fresh water environments.

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#### **TANK CULTIVATION OF *ULVA* (CHLOROPHYCEAE) IN FLORIDA**

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*Ulva* spp. has been grown in outdoor tank cultivation at Harbor Branch for the last 25 years. Initially, it was difficult to maintain individual strains throughout the year due to loss of biomass as a result of reproduction under the relatively high water temperature found

during the summer. A search for potential warm-water strains was conducted in nearby marine waters and several promising strains were isolated into laboratory cultures. Growth experiments in the laboratory and in greenhouse cultures resulted in the selection of strains that could grow year-round, with little or no losses due to reproduction. There is significant physiological variability within a species; the relationship of growth to environmental conditions (e.g., temperature and salinity) is strain dependant. Productivity rates in intensive "tumble" cultivation (high aeration and seawater turnover) are typically 25–30 g dry weight/m<sup>2</sup>/d, but can reach 35 g dry weight/m<sup>2</sup>/d, averaged over the annual cycle. Currently, *Ulva* is being cultivated in this system and used either fresh or as a dried powder, as feed for animals, either to experimental animals in the lab or for inclusion in diets of marine fish. *Ulva* is a strong candidate in "integrated aquaculture", which is the focus of renewed interest in recent years, due to *Ulva*'s high rates of growth and nutrient uptake, and its excellent nutritional quality for many marine herbivores.

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#### **AN UPDATED CHECKLIST OF THE SEAWEEDS AND SEAGRASSES OF OREGON**

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The recent checklist of the marine macrobenthic marine algae and seagrasses of Oregon (Hansen 1997) was based primarily on the literature. Although it revealed a depauperate flora of only 387 species, the low count was thought to be due in part to a lack of adequate floristic investigation in this area. To help correct this problem, a database of the existing collections of marine algae from Oregon was initiated. To date, 7500 specimens from 10 major herbaria have been cataloged. New records from these archived specimens and from some new collections have increased the species count by over 50. Further cataloging and collecting should reveal additional new records for the state, but it is unlikely that the predominantly exposed, sand-scoured coastline will ever be found to be as species rich as the more habitat diverse neighboring states of Washington and California. In addition to the updated checklist, the poster will include the early collection history of the state and some discussion of the problematic taxa.

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#### **ENVIRONMENTAL CHANGE, INTERSPECIFIC INTERACTIONS, AND SHIFTING ALGAL DISTRIBUTIONS ON ROCKY SHORES**

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Because intertidal organisms live close to their environmental tolerances, intertidal species are considered early harbingers of the effects of global change. Using a combination of spatial environmental gradients and long-term datasets, I examine the likely consequences of global warming on the distribution of *Mazzaella parksii* (formerly *Mazzaella cornucopiae*), a turf-forming red alga. On Tatoosh Island, WA, *M. parksii* is restricted almost entirely to north-facing surfaces. On south-facing slopes, the upper limit of *M. parksii*, which is set by abiotic stress, occurs much lower on the shore. However, its lower limit, which is set by herbivores, does not vary with aspect. Because its upper and lower limits are independent across the aspect gradient, *M. parksii* is effectively “squeezed out” of the system as one moves from a north-facing to a south-facing slope. This general pattern is observed at several spatial scales across gradients of wave exposure and air temperature. The vertical distributional patterns of *M. parksii* also shift across temporal gradients. Over the past 25 years, the upper limit of *M. parksii* on Tatoosh Island has shifted downshore by approximately 25 cm. Most of this change was driven by three consecutive harsh summers in the mid 1990’s. These temporal patterns, combined with the insight gained from spatial patterns, suggest that *M. parksii* may go locally extinct in many parts of its range as its upper and lower limits converge. Furthermore, because vertical distributional shifts are linked to stochastic climatic effects, temporal change in marine algal populations may be rapid and unexpected.

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**NUCLEUS-ENCODED, PLASTID-TARGETED GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE (GAPDH) INDICATES A SINGLE ORIGIN FOR CHROMALVEOLATE PLASTIDS**

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Plastids (the photosynthetic organelles of plants and algae) ultimately originated through an endosymbiosis between a cyanobacterium and a eukaryote. Subsequently, plastids spread to other eukaryotes by secondary endosymbioses that took place between a eukaryotic alga and a second eukaryote. Recently, evidence has mounted in favour of a single origin for plastids of apicomplexans, cryptophytes, dinoflagellates, haptophytes, and heterokonts (together with their non-photosynthetic relatives, collectively termed chromalveolates). As of yet, however, no single molecular marker has been described which supports a common origin for all of these plastids. One piece of the evidence for a single origin of chromalveolate plastids came from plastid-targeted

glyceraldehyde-3-phosphate dehydrogenase (GAPDH), which originated by a gene duplication of the cytosolic form. However, no plastid GAPDH has been characterized from haptophytes, leaving an important piece of the puzzle missing. We have sequenced genes encoding cytosolic, mitochondrial-targeted, and plastid-targeted GAPDH proteins from a number of haptophytes and heterokonts, and found the haptophyte homologues to branch within the strongly supported clade of chromalveolate plastid-targeted GAPDH genes. Interestingly, plastid-targeted GAPDH genes from the haptophytes were more closely related to apicomplexan genes than was expected. Overall, the evolution of plastid-targeted GAPDH reinforces other data for a red algal ancestry of apicomplexan plastids, and raises a number of questions about the importance of plastid loss and the possibility of cryptic plastids in non-photosynthetic lineages such as ciliates.

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**NUTRIENT RESPONSES OF HARMFUL ALGAL BLOOMS IN SOUTH CAROLINA BRACKISH LAGOONAL SYSTEMS**

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The widespread occurrence of harmful algal blooms (HABs) in South Carolina brackish lagoons has recently been documented. Over the last 2 years, dense blooms of raphidophytes, dinoflagellates, cyanobacteria, and prymnesiophytes were commonly observed, mostly in storm water retention ponds associated with housing developments or golf courses. As part of an effort to understand the physiological ecology of these blooms, bioassays were performed testing the effects of nutrients on HAB population growth. The first bioassay was conducted on a mixed bloom of *Heterosigma akashiwo* and *Chattonella verruculosa*, and the second on a mixed bloom of *Fibrocapsa japonica*, *Karlodinium micrum* and *C. verruculosa*. The growth of all species but *F. japonica* could be stimulated by the addition of DIN or DON substrates, including nitrate, ammonium, urea, and glycine. *F. japonica* was only stimulated by the addition of a fertilizer (Miracle Grow™) or an inhibitor of photosynthesis (DCMU). Stimulation by the fertilizer, but not N or P substrates, suggests that this response was related to trace metal (e.g. Fe) use. Growth stimulation by DCMU may reflect a high heterotrophic capability or insensitivity of *F. japonica*'s photosynthetic system to this inhibitor. The results suggest that the nutritional versatility of these species may contribute to their widespread occurrence and

ability to form blooms in these low flow, nutrient- and organic-rich systems. Also, the responses of *F. japonica* infer that contaminant loading (e.g. herbicides with similar effects as DCMU) should be considered as a selective factor in HAB initiation or maintenance.

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### ALGAL BIOREMEDIATION OF MINE WASTE TAILINGS BY APPLICATION OF MOSS PROTONEMA

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Moss was discovered growing on mine waste tailings with high heavy metal content and low pH at the Badger State Mine near Butte, MT. It has been proposed that inoculation of plots with moss protonema, a stage of moss development, will produce a microbial mat. This process is fairly straightforward in mesic environments, but the aridity, acidity and high metal content of the tailings present some difficulty with the establishment of moss protonema or even algae. Consequently, a hydrophilic polymer and nutrients will be applied in addition to the protonema. This combination may assist in the retention of moisture to ensure the growth of the mature moss and other pioneer organisms. Soil from the mine tailings will be analyzed for evidence of cyanobacterial and other algal diversity. Benefits of cyanobacteria growth include their ability to fix nitrogen for use by other plants and production of photosynthates that leak organic carbon into the soil at a rate that can feed the heterotrophic microbes found in the microbial mat. The combination of algae, fungi, bacteria, protists and moss in a self-sustaining microbial mat may help to stabilize the soil and create a hospitable environment for the colonization of vascular plants. Formation of a microbial mat in this xeric environment of mine waste tailings with the use of a hydrophilic polymer may be an ideal solution for the stabilization of mine wastes.

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### PATTERNS OF REPRODUCTION AND MORPHOLOGICAL VARIATION IN SOUTHERN CALIFORNIA POPULATIONS OF THE LOWER INTERTIDAL KELP *EGREGIA MENZIESII* (TURNER) ARESCHOUG

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*Egregia menziesii* is a complex kelp with an adult morphology that varies considerably across its geographic range. Fertility in *Egregia* also varies geographically. We studied intertidal populations of *Egregia* at three southern California sites to determine spatial and temporal patterns of reproduction, recruitment, and morphology. Growing axes of *Egregia* were collected monthly to quantify sporophyll production and lateral blade morphology. Band transects were surveyed quarterly (2001) and monthly (2002) to determine temporal patterns of sporophyte recruitment. Sporophylls were found throughout the year, but sporophyll densities showed a strong peak during winter periods of colder seawater temperatures and shorter daylengths. Sporophytes recruited almost exclusively from March to July, suggesting a gametophyte period of <5 months. Lateral blade morphologies varied in a consistent pattern; thalli initially developed spatulate blades but then produced increasing numbers of filiform laterals as axes grew. Thalli in habitats populated by higher grazer densities had shorter axes, dominated by spatulate blades, while those growing under low grazing pressure exhibited longer fronds and more filiform laterals. Differences in lateral morphology had effects on thallus productivity and susceptibility to grazers. Filiform laterals had higher light-saturated photosynthetic rates than spatulate laterals and a 1-m long frond with filiform laterals was estimated to be 12% more productive than one with spatulate laterals. Kelp snails, but not urchins, consistently chose spatulate over filiform laterals, this suggests that *Egregia thalli* dominated by spatulate laterals are more susceptible to grazing molluscs, while lateral morphology has no effect on *Egregia's* susceptibility to urchins.

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### A NEW PARADIGM FOR THE APHANIZOMENON BLOOM PROBLEM IN UPPER KLAMATH LAKE

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Upper Klamath Lake (UKL) is the largest lake in Oregon (area 287 km<sup>2</sup>, avg. depth 4.2 m). It is naturally eutrophic and regularly suffers nuisance summer blooms of cyanobacteria, principally *Aphanizomenon flos-aquae* (AFA). Sediment coring studies show that AFA was absent or minimal until about 1880 when a steady increase began, culminating in the blooms of recent decades. These studies show concomitant increases in sediment N (~20%) and P (~50%) along with shifts in the algal flora indicating

increased eutrophication. These changes correlate with increased human impacts, such as deforestation, construction, roadbuilding etc., and especially the ditching, diking and draining of adjacent wetlands for conversion to agriculture. Agricultural nutrient runoff, especially P, has been often cited as the cause of the AFA blooms, and most attention has been focused on the dynamics of UKL during the summer bloom. We propose that a more significant factor may be the loss of early-season suppression of AFA because of the loss of the lake-associated wetlands, which originally constituted 42% of the lake area, and which have declined in area by 66.3% since the late 1800's. The melting of snow and ice in the spring would flush into the lake a surge of wetland plant decomposition products, most significantly organic acids and humic substances. We propose that formerly these wetland effluents caused a complex of effects on lake pH, solar UV transparency, photochemical interactions, nutrient availability, and *Daphnia* grazing dynamics, which would have combined to prevent the development of any AFA bloom.

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#### **CHARACTERIZATION OF THE PHYTOPLANKTON IN LOW ORDER STREAMS OF SOUTH MISSISSIPPI**

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Twenty-six collection sites located in 24 headwater creeks that originate and drain from the Camp Shelby Training Facility in South Mississippi were sampled eight times between November 1998 and October 2000. Seventeen physical and chemical properties of the creeks were measured. Phytoplankton abundance and richness were recorded. Relationships between abiotic and biotic variables were analyzed. Nitrogen, phosphorus, and potassium for most creeks except Weldy creek remained low according to the EPA water quality criteria for streams and aquatic life. Total solids, conductivity, turbidity, and fecal coliform bacteria increased after local precipitation. Shelby creeks were characterized by having low phytoplankton abundance and high genera richness. Algal concentrations were under 300 org/l. Phytoplankton genera richness was influenced by water temperature and flow. The phytoplankton was represented by 99 genera belonging to six divisions. The Chlorophyta and the Chrysophyta accounted for 81% of the genera richness. The diatoms *Navicula*, *Tabellaria*, *Nitzschia*, *Surirella*, *Eunotia*, *Pinnularia* and the green algae *Closterium*, *Mougeotia*, and *Ankistrodesmus* were recurrent members of the algal community.

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#### **USE OF MOLECULAR PROBE TO DETECT TASTE/ODOR-CAUSING CYANOBACTERIA IN THE PHOENIX DRINKING WATER DISTRIBUTION SYSTEM**

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Tastes and odors caused by 2-methyl-isoborneol (MIB) and geosmin occur frequently in drinking water supply sources and aquaculture environments. Cyanobacteria often are implicated as culprit organisms responsible for production of these compounds. To develop cost-effective and efficient mitigation measures to prevent or reduce the off-flavors requires a sensitive, specific method with forecasting ability to detect the occurrence and distribution of the culprit species in a water system. Conventional biological (e.g., microscopy, isolation and cultivation of algae) and chemical (e.g., gas chromatography and mass spectrometry) methods are useful, but are usually incapable of providing predictive information. In this report, we introduce a new molecular fingerprinting technique for probing for the presence of cyanobacteria that have the genetic potential to produce MIB and geosmin. This method involves denaturing gradient gel electrophoresis (DGGE) analysis of PCR-amplified 16S rDNA fragments. When analyzed by this method, the phylogenetic composition of benthic cyanobacterial assemblages differed substantially among ten permanent sampling sites along the Arizona Canal. The Arizona Canal is an open channel conveying water to four water treatment plants providing drinking water to over two million people in the Phoenix metropolitan area. Clear relationships were observed between specific DNA fingerprints, and episodes and intensity of MIB/geosmin production in specific sections of the canal. This suggests that the method could serve as a sensitive and reliable molecular method for early detection and forecasting of taste/odor episodes in water environments.

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#### **PROTEOMICS OF HAEMATOCOCCUS PLUVIALIS: NEW OPPORTUNITIES FOR STUDY OF GENOMICS OF A NON-SEQUENCED SPECIES**

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The green alga, *Haematococcus pluvialis*, has become a model organism for commercial production of the high-value carotenoid astaxanthin. *H. pluvialis* has also drawn significant scientific attention because fundamental biological questions relating to the massive cellular accumulation of astaxanthin have to be addressed in order to improve the yield and quality of the algal biomass. However, research has been impeded by the lack of molecular background information on this non-sequenced species. A combination of classical biochemistry with a state-of-the-art proteomic approach was used to address these questions. This was possible by taking advantage of information already available for homologous genes/gene-products in organisms whose genomes have been sequenced. The approach involved isolation of subsets of the proteome from subcellular compartments/organelles of an organism by one- or two-dimensional electrophoresis (1-DE or 2-DE) and their identification by N-terminal sequencing and peptide mass fingerprinting (PMF), involving matrix-assisted laser desorption/ionization and time-of-flight (MALDI-TOF) mass spectrometry coupled with bioinformatics. Based upon the information obtained from the combined methods, expression and physiological functions of specific genes/encoded proteins may be deduced. Examples include profiling of cell wall proteins, biogenesis and protein composition of lipid bodies, and expression patterns of soluble proteins under stress conditions. Advantages and limitations of the method for non-sequenced organisms and for cross-species protein identification will also be discussed.

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#### AN EXAMINATION OF THE PERIPHYTIC ALGAE IN THE SHEYENNE RIVER, NORTH DAKOTA, USA, AND THE FACTORS INFLUENCE THE COMMUNITY STRUCTURE

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The Sheyenne River is a third order stream in central and eastern North Dakota. The algae in this river had not been previously studied. For this study, periphytic algal samples were collected Fall 1997, April–November 1998, and May–October 1999. Two hundred sixteen periphytic algal taxa were identified. These taxa included 137 Bacillariophyceae, 33 Cyanophyta, 40 Chlorophyta, 8 Xanthophyceae, and 3 Eugleno-

phyta. Several species of algae found in the river appear to be previously undescribed taxa. Two of these organisms were described as new species, *Nitzschia sheyennensis* and *Nitzschia dakotensis* (Bacillariophyceae). The distribution of the periphytic algae was correlated to changes in the selected environmental variables using canonical correspondence analysis (CCA) and multi-dimensional scaling (MDS). The statistically significant environmental variables were nitrite-nitrate, pH, arsenic, potassium, magnesium, manganese, and total dissolved solids. A reservoir, Lake Ashtabula on the river was formed by a dam in 1954. In the initial statistical analysis, the effects of this reservoir on the periphytic algae downstream were so great that an upstream/downstream covariable had to be used. Further statistical analysis was used to determine that this effect could be correlated to changes in the nutrient levels rather than hydrologic changes.

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#### THE USE OF ALGAE TO TREAT WASTEWATER IN THE BERKELEY PIT

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The Berkeley Pit in Butte, Montana is currently filled with 35 billion gallons of water, with 6 million gallons being added daily. The water in the pit contains a number of dissolved metals and other elements. These elements include: aluminum, iron, manganese, zinc, copper, cadmium, magnesium, nickel, calcium, sulfur, and silicon. Also, over 40 different types of small organisms live in the contaminated water. The purpose of this experiment is to examine the uptake potential of elements from Berkeley pit water by highly concentrated algal samples. The algae used are called *Chlamydomonas acidophila*. *Chlamydomonas acidophila*'s optimum pH is between 3.5 and 4.5 and it may be capable of metal uptake by two mechanisms: absorption and adsorption. Adsorption and absorption are good alternatives to traditional wastewater treatments because algal cells are a naturally abundant renewable resource that can be cheaply produced. Algal cells are also highly selective in terms of removal and recovery of specific heavy metals, they do not require reagents, which typically cause disposal and space problems, and they require a relatively low initial capital investment with low operating costs. The experimental procedure involved using modified acid medium to grow algae from a stock solution to a concentration of  $1.6 \times 10^8$  algae cells/mL. The growth medium was then washed from the algae cells. Three initial

samples of sterile filtered pit water (SFPW) were set aside as a control. Then, concentrated algae cells were added to three centrifuge tubes. Next, SFPW was added to 3 tubes with algae and three tubes without algae. After 1 hour, a 5 mL sample was taken from each of the centrifuge tubes. After 24 hours, another 5 mL sample was taken from each of the centrifuge tubes. Inductively Coupled Plasma (ICP) analysis was performed on all the samples. The results from the initial SFPW were compared with those samples containing algae for 1 hour and 24 hours. Total metal uptake in 24 hours was roughly 7.81%.

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#### DIVERSITY OF *SCENEDESMUS* FROM ITASCA STATE PARK, MINNESOTA

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The diversity of *Scenedesmus* and *Scenedesmus*-like taxa from Itasca State Park, Minnesota was assessed using light microscopy and molecular techniques. Thirty isolates from various ponds and lakes in Itasca State Park were examined. Light microscopy showed many similarities in morphology among isolates, but PCR-RFLP analysis of the ribosomal ITS region from these isolates revealed twenty different types. A previous study from Itasca State Park using only light microscopy found only six taxa of *Scenedesmus*; however, our results suggest that there is much greater diversity than previously suspected. DNA sequences of the 5.8S ribosomal subunit and the ITS-2 region from our isolates are presently being determined and will be used to assess this diversity in greater detail.

## 75

#### THE ROLE OF FEEDING IN GROWTH AND PHOTOSYNTHESIS OF *MYRIONECTA RUBRA*

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*Myrionecta rubra* (= *Mesodinium rubrum*) is an obligate phototroph that possesses plastids, mitochondria and sometimes nuclei of cryptophyte algae. Plastids and nuclei have been observed to increase in *M. rubra* when feeding. The current study was conducted in order to better understand the relationship between feeding and photosynthetic physiology in *M. rubra*. During the experiment, cryptophytes were introduced for 2 consecutive growth periods (14 d) and the cultures were then starved during 4 additional periods. Photosynthesis-irradiance (PE) response curves were measured twice during each growth period (days 7 and 14), and the parameters alpha and

Pmax were measured and normalized to cell and chlorophyll (chl) concentrations. In both treatments, growth rate and nuclei/cell increased significantly when fed ( $p < 0.05$ ), and then declined over time. Cell volume increased substantially in the HL treatment when in the presence of prey ( $p < 0.05$ ), but otherwise varied little. In both treatments, chl/cell and Pmax(-cell) increased after feeding and then declined after several weeks in the absence of prey. Absolute changes in these parameters were greater in the LL treatment. In both treatments Pmax(chl) increased over the study. As observed previously, *M. rubra* appears to possess a limited capacity for producing pigments. While these results illustrate some dependency of photosynthesis and growth in *M. rubra* upon ingestion of cryptophyte prey, they also display that the ciliate can use and sustain plastids like a phototroph.

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#### THE *PNEUMOCYSTIS* SAM:SMT, AN ATYPICAL FUNGAL ENZYME PROTEIN

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*Pneumocystis*, an opportunistic fungal protist, causes a type of pneumonia in immunocompromised individuals such as AIDS patients. Rat-derived *P. carinii* and human-derived *P. jirovecii* contain a large number of sterols with C-24 alkyl groups. S-Adenosyl-L-methionine:sterol C-24 methyl transferase (SAM:SMT) is the enzyme that transfers methyl groups from SAM to the C-24 position of the sterol side chain. An alkyl group at the C-24 sterol side chain position appears to be essential for the organism to proliferate. Thus SAM:SMT, which is absent in mammals, is an attractive target for chemotherapeutic attack against the pathogen. The *P. carinii* erg6 gene that codes for SAM:SMT has been sequenced, cloned, and the protein expressed in *E. coli*. Since bacteria do not synthesize sterols, and do not have SAM:SMT, the *P. carinii* erg6 gene product expressed in *E. coli* would only transmethylylate exogenously provided sterol substrates. The *P. carinii* recombinant SAM:SMT is unique because lanosterol, a central intermediate in sterol biosynthesis, is its preferred substrate for enzyme activity. Most SAM:SMT from other organisms do not bind lanosterol and prefer other sterol substrates produced from lanosterol. Furthermore, it appears that this unusual *P. carinii* SAM:SMT can also methylate cholesterol, which is readily scavenged from the lungs of its rat host. The recombinant enzyme protein is being purified by affinity chromatography techniques, which will be used to obtain definitive structural analyses of the sterol compounds formed by the enzyme reaction

using different sterols substrates and allow detailed structural analysis of this unusual SAM:SMT enzyme protein.

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### CRYPTIC SPECIATION OF *PERIDINIUM LIMBATUM*

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Cosmopolitanism vs. endemism of microbial diversity in water has been debated for a long time. Finlay (2002) argued that reported similarities in microbial biodiversity in different lakes and oceans support cosmopolitanism of microorganisms. Lee and Patterson (1998) suggested that many endemism studies are flawed due to underrepresentation of taxa. On the other hand, Theriot and Stoermer (1984) showed that *Stephanodiscus niagarae*, the centric diatom once thought to be a cosmopolitan species, actually consists of four slightly different species. Mating tests of *Paramecium aurelia* revealed 14 sibling species, which are morphologically almost identical (Sonneborn, 1957). Here, I present evidence for the cryptic speciation of *Peridinium limbatum* from two water bodies (Crystal Lake and Crystal Bog) that are near each other but chemically distinct. We observed that the two *Peridinium limbatum* had different responses to the same growth medium. Previous works at the Microbial Observatory program showed that their seasonal population dynamics were also different; while a summer bloom of Crystal Bog *Peridinium limbatum* was correlated with a dramatic decrease in phytoplankton diversity, and an extreme drop in bacterial diversity, a bloom of Crystal Lake *Peridinium limbatum* was not. To see the genetic variations in this study, I sequenced internal transcribed spacer region 1 and 2 from the two *Peridinium limbatum*. The sequence variations were 8.53% in ITS1 and 8.06% in ITS2, which were much larger than within variations. In another ongoing study that I expect to complete before the meeting, I've been testing their possible allelopathic differences on Cryptomonas.

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### A COMPARISON OF FOUR NATIVE PORPHYRA SPECIES FROM COASTAL NEW ENGLAND IN RESPONSE TO AMMONIUM AND TEMPERATURE IN SMALL TANK SYSTEM

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*Porphyra* is one of the world's most valued maricultured seaweeds. The effects of ammonium concentration and temperature on growth and tissue nitrogen content of *Porphyra* from coastal New England were studied in 50 liter tanks to produce critical information for the development of a land based aquaculture system. Four Northwest Atlantic *Porphyra* species: *P. leucosticta*, *P. amplissima*, *P. linearis* and *P. umbilicalis*, were cultivated for two to three weeks at saturating light intensities (100–150 $\mu$ Em-2s-1) and six combinations of ammonium (25 and 250 $\mu$ M) and temperature: 10, 15 and 20 $^{\circ}$ C. The specific growth rate (SGR) of all species under most temperature conditions was significantly higher at 250 $\mu$ M than at 25 $\mu$ M ammonium. *P. leucosticta* showed higher growth rate at 10 and 15 $^{\circ}$ C (10%d-1 at 250 $\mu$ M versus 9 – 9.5%d-1 at 25 $\mu$ M) in the first week. The SGRs of *P. linearis* in the second week and *P. umbilicalis* in the first week were the highest at 10 $^{\circ}$ C (16 and 10%d-1 at 250 $\mu$ M versus 12 and 8%d-1 at 25 $\mu$ M, respectively). The SGR decreased with increasing temperature. In contrast, *P. amplissima* showed the highest SGR at 20 $^{\circ}$ C (9%d-1 at 250 $\mu$ M versus 7%d-1 at 25 $\mu$ M) in the first week, and the SGR decreased at lower temperatures. Nitrogen concentration in tissue of *P. leucosticta* was significantly higher at 250 $\mu$ M than at 25 $\mu$ M. The rapid nutrient assimilation and fast growing ability makes *Porphyra* an excellent candidate for bioremediation and integrated aquaculture.

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### SYSTEMATIC REVISION OF PHAEOSTROPHION IRREGULARE (PHAEOPHYCEAE) AND PROPOSAL OF A NEW FAMILY PHAEOSTROPHIONACEAE

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*Phaeostrophion irregulare* S. & G. is distributed along the Pacific coast of NW America, and has been placed in the family Punctariaceae, order Dictyosiphonales (or Ectocarpales s.l.), based on morphological resemblances. Culture studies have indicated a direct life history, with erect thalli forming unilocular and/or

plurilocular sporangia. However, the occurrence of a perennial prostrate system (holdfast), presence on the holdfast of marginal meristematic cells, and lack of pyrenoids in the chloroplasts, suggest that this taxonomic assignment is questionable. Our TEM observations confirm the lack of pyrenoids in the chloroplasts. The occurrence of reductive divisions in the unilocular sporangia was confirmed by comparing the DNA content in each nucleus using a fluorescent stain, but both unilocular and plurilocular sporangia-bearing erect thalli showed diploidy. Molecular phylogenetic analysis using *rbcL* (chloroplast) and 18S rDNA (nuclear) gene sequences reveals that *Phaeostrophion* is not included in the clade of Ecotocarpales s.l., but diverged relatively early in the evolution of Phaeophyceae, forming a clade with Sphacelariales and Syringodermatales, which share the apical/marginal meristematic mode of growth. Although we propose a new family Phaeostrophionaceae to accommodate *Phaeostrophion*, we suspend judgment of the appropriate taxonomic treatment at the ordinal level.

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**PURIFICATION AND CHARACTERIZATION OF MALE AND FEMALE GAMETE RECOGNITION MOLECULES OF A MARINE RED ALGA *AGLAOTHAMNION OOSUMIENSE***

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In red algae, fertilization begins with gamete-gamete contact between the trichogyne cell wall of the female carpogonium and spermatial coverings. During the fertilization in *Aglaothamnion oosumiense*, reproductive cells interact with each other through sex specific adhesion molecules on the surface of spermatia and trichogyne. The gamete binding is highly selective suggesting the presence of recognition factors along their surfaces. In the previous studies, we have reported that spermatial binding to trichogynes of a red alga, *Aglaothamnion oosumiense* is mediated by a lectin-carbohydrate complementary system. Spermatial binding to trichogynes was inhibited by preincubation of trichogynes with N-acetyl-D-galactosamine and D-glucose and hence lectins specific to these sugars were expected to be present on the surfaces of trichogyne cell wall. We have isolated a new lectin from *Aglaothamnion oosumiense* by the use of agarose bound N-acetyl-D-galactosamine affinity chromatography and named it as rhodobindin. Rhodobindin agglutinated human erythrocytes as well as spermatia of *Aglaothamnion oosumiense*. The agglutinating activity of this lectin was inhibited by N-acetyl-D-galactosamine and N-acetyl-D-glucosamine. SDS-PAGE results showed that this lectin may be monomeric. The molecular weight was determined as 21,876 dalton by

matrix-assisted laser desorption ionization (MALDI) mass-spectrometry. N-terminal amino acid sequence of the lectin was analyzed and revealed to have no identity with those of known proteins. The complementary male glycoprotein was also isolated and purified by the use of SBA-agarose affinity chromatography. The subtractive cloning was carried out to characterize the recognition molecules.

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**RECENT INTRODUCTION OF *POLYSIPHONIA MORROWII* (CERAMIALES, RHODOPHYTA) TO PUNTA ARENAS, CHILE**

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*Polysiphonia morrowii* (Rhodomelaceae, Rhodophyta) is abundant intertidally in spring in the Northwest Pacific. The species occurs in a large part of the North Sea and in Thau Lagoon, France, showing disjointed distributions, where the alga has been probably introduced recently. Using plastid protein-coding *rbcL* sequence data from specimens of *P. morrowii* and putatively related taxa, collected in Korea, Japan and Far-east Russia, and Chile, we tested the taxonomic position of each taxon. Pairwise divergence and topology of the sequences indicate that the Chilean taxon was identical to *P. morrowii* and was clearly separated from other related taxa from Chile. The results suggest that *P. morrowii* is recently anthropogenically introduced from the Northwest Pacific to Chile by boat or by transport with aquacultured organisms. Detailed observations of field-collected material will probably enable a more realistic evaluation of distribution of *P. morrowii* to be made in the Pacific Ocean.

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**TAXONOMIC REVISION OF *SPHAEROTRICHIA DIVARICATA* (C. AG.) KYLIN (ECTOCARPALES S.L., PHAEOPHYCEAE), WITH A REAPPRAISAL OF *S. FIRMA* (GEPP) A. ZINOVA FROM THE NORTHWESTERN PACIFIC**

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Specimens of Sphaerotrichia in Japan can be classified into two morphotypes based on mode of branching and anatomy: 1) specimens having an obvious (traceable) main axis associated with more first-order branches, and the distance between the branches is relatively constant; 2) specimens that branch more or less divaricately and the distance between branches tends to be shorter in the distal portion. Molecular analyses of North Atlantic and North Pacific Sphaerotrichia using ribosomal RNA (ITS1-5.8S-ITS2 and IGS regions between 26S and 5S rDNA) and Rubisco (almost complete *rbcL* gene and the spacer between *rbcL* and *rbcS*) gene sequences revealed that there are two major genetic groups (i.e. Group-1 and Group-2), whereas the sequence length variation in ITS1 suggested subdivisions of Group-2 into two subgroups (Group-2Atlantic and Group-2Pacific). Specimens belonging to Group-1 were generally epilithic, and morphologically corresponded to *Sphaerotrichia firma* (Gepp) A. Zinova. Specimens belonging to Group-2 were epiphytic or epilithic and morphologically corresponded to *S. divaricata* (C. Ag.) Kylin.

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#### COMPARATIVE ANALYSIS OF VERTICALLY MIGRATING *EUGLENA VIRIDIS* POPULATIONS IN TIDAL AND NON-TIDAL BENTHIC ENVIRONMENTS

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Benthic populations of *Euglena viridis* exhibit vertical migration behavior on high energy intertidal beaches and along the sand banks of freshwater streams. This study examines similarities and differences in the migratory behavior and cell morphology of populations of *E. viridis* inhabiting Scripps Beach, La Jolla, California and Coble Brook, Burlington, North Carolina. The timing of migration was measured by counting the number of cells in samples collected from the sediment surface throughout the day. Sediment cores were extracted and sectioned to determine the vertical distribution of the population. Neutral density filters and opaque canisters were used to shade the substratum to 56%, 22%, 2%, and 0% of incident irradiance ( $I_0$ ) to examine the effect of light on cell morphology and migratory behavior. On intertidal beaches, *E. viridis* exhibited a tidal rhythm in vertical migration with cells migrating below the sediment surface at night (>15 cm) and during daytime high tides. In this habitat, the upward migration response was enhanced at irradiances lower than 100%  $I_0$  but cell morphology was not altered by shading. On the banks of freshwater

streams, *E. viridis* exhibited a diurnal migratory rhythm with both tear-drop and spherical morphologies observed throughout the day. The population was most concentrated at the surface around solar noon and at night it was located between 1 and 2 cm below the surface. Shading did not enhance upward migration but it did affect cell morphology. These results will be interpreted in the context of the dominant selection pressures in each environment.

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#### LINKING ENVIRONMENTAL FORCING, KELP FOREST HABITAT DYNAMICS, AND COMMUNITY STRUCTURE IN THE NORTHEAST PACIFIC

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Habitat-forming species of large brown macroalgae (e.g., kelps) often differ from associated benthic species in resource requirements, sources of disturbance, and dispersal ability. Differences in environmental drivers and demographic processes may cause these habitats to fluctuate at spatial and temporal scales that differ from the "optimal" scale that would promote maximum abundance of any particular associate species. As a result, the spatiotemporal dynamics of habitat may exert important effects on benthic community structure and composition. To quantify the spatial and temporal dynamics of giant kelp (*Macrocystis pyrifera*), a key habitat-former in the NE Pacific, I analyzed a 34-year monthly time series of estimated canopy biomass spanning ~1500 km of coastline (7° of latitude) and digital maps of annual maximum canopy cover. Canopy biomass varied interannually at dominant periods of 4–5 y, 11–13 y and ~20 y, and spatial scales ranging from local (~30 km) to mesoscale (~100–150 km) and regional (~330 km). Temporal dynamics were strongly related to basin-scale climate fluctuations (El Niño-Southern Oscillation, Pacific Decadal Oscillation) and spatial patterns were correlated with coastline geomorphology. Digital canopy maps reveal that changes in biomass are associated with shifts in the spatial structure of the kelp habitat. Long-term subtidal community monitoring data from areas with markedly different spatial and temporal scales of kelp forest habitat structure reveal a complex but important influence of habitat dynamics on the distribution of life histories within kelp-associated communities. Future changes in the dynamics of Pacific climate fluctuations may have important implications for kelp forest community structure.

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### REGENERATION OF PROTOPLASTS FROM DISINTEGRATED CELLS OF THE MULTI-CELLULAR MARINE GREEN ALGA *MICRODICTYON UMBILICATUM*

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Protoplast regeneration from extruded cytoplasm of the multi-cellular marine green alga *Microdictyon umbilicatum* (Velley) Zanardini (Cladophorales, Anadyomenaceae) was investigated. The early process of protoplast formation is comprised of two steps: agglutination of cell organelles into protoplasmic masses followed by generation of a temporary enclosing envelope around them. Agglutination of cell organelles was mediated by a lectin-carbohydrate complementary system. Three sugars, D-galactosamine, D-glucosamine, and  $\alpha$ -D-mannose, inhibited the agglutination process, and three complementary lectins for the above sugars, peanut agglutinin, *Ricinus communis* agglutinin and concanavalin A, bound to the surfaces of chloroplasts. Agglutination assay using human erythrocytes showed the presence of lectins specific for the above sugars in the algal vacuolar sap. The lectin has been purified by the use of D-mannose agarose affinity column. Its Molecular weight was shown to be 36,000 dalton by SDS-PAGE gel electrophoresis. When the basic regeneration process was accomplished, the cells chose one of two developmental strategies; about 70% of one-celled protoplasts transformed into reproductive cells within two weeks after wounding, while others began cell division and grew into typical *Microdictyon* plants. Quadriflagellate swimmers were liberated from the reproductive cells, and they germinated into mature plants

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### CHLOROPLAST GENOME PHYLOGENETICS: FACTS AND OPINIONS

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Recent progress in sequencing genomes from primary and secondary plastids provided various data including genomic complexities, gene sequences, gene complements, gene cluster organizations and rearrangements as well as secondary gene losses/gene relocations. They are considered to reflect evolutionary traits at different phylogenetic levels. However, current views of the validity of individual data sets are controversial and sometimes appear to reflect opinions rather than facts. Here I will present the plastid genomes of the Xanthophyte *Vaucheria bursata* and the brown alga *Dictyota dichotoma* and

compare these genomes with those of other algae containing secondary plastids of rhodophytic origin. Gene complement analysis will focus on differential gene losses across evolutionary lineages and gene cluster rearrangements as a consequence of numerous inversions unknown from land plants. It will be discussed whether rearranged gene clusters common to two lineages, to the exclusion of others, may reflect parsimonious events or simply resulted from random independent processes. Although specific characters are encountered among all plastid genomes sequenced to date, the plastid genomes of *Vaucheria* and *Dictyota* confirm the presence of a basal set of 46 protein coding genes common to all plastid genomes of algae and metaphytes that have been used to infer a monophyletic origin of primary plastids and their evolutionary offsprings.

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### ALGAL BIOREMEDIATION OF EUTROPHIC EFFLUENTS IN SMALL SCALE INTEGRATED AQUACULTURE SYSTEMS

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Non-point source eutrophication of coastal waters is a significant problem that may be exacerbated locally by effluent from aquaculture operations. *Porphyra* spp. grow and assimilate nutrients rapidly, making them good candidates for eutrophication abatement via systems of integrated aquaculture. I summarize our work examining the bioremediatory performance (growth rate, nutrient assimilation, tissue N and pigment content) of four U.S. and three Asian *Porphyra* species as functions of N concentration and source (nitrate vs. ammonium). The Northeast U.S. species *P. amplissima* is the best performing local bioremediator (maximum growth rate and tissue N = 24% d<sup>-1</sup>, 5.2% DW, respectively), comparing well with *P. yezoensis*, an economically important species in Asia. When tissue remained non-reproductive, *P. amplissima* growing in 300  $\mu$ M ammonium removed 99–100% of N but only about 50% of P (fed 10:1 molar N:P ratio). We have begun investigating the relationship between stocking density and yield, and will begin demonstration scale tests of the mesoscale system.

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**PRELIMINARY OBSERVATIONS ON THE IDENTITY OF THE RED ALGAL GENUS CALOGLOSSA (DELESSERIACEAE, CERAMIALES) IN THE NORTHWESTERN GULF OF MEXICO**

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Phylogenetic analyses inferred from *rbcL* sequences indicates that specimens of *Caloglossa* (Harvey) G. Martens collected from Vermilion Bay, Louisiana and Galveston, Texas, reported as *C. lepriaurii* (Montagne) G. Martens (1869) constitutes a separate taxon. Studies are presently being conducted to compare this northwestern Gulf of Mexico taxon with material of *C. intermedia* Kamiya et West in Kamiya et al. (2000, J. Phycol. 36:411-420) from the Western Atlantic coast and with *C. apomeiotica* West et Zuccarello in West et al. (1994, Bot. Mar. 37:381-390) from Baja California, Mexico.

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**MORPHOLOGICAL AND MOLECULAR CHARACTERISATION OF FOUR NOSTOC SPECIES ISOLATED FROM DESERT SOILS**

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Four unusual strains of *Nostoc* species from desert soils of the western United States were chosen for morphological and molecular characterization. The morphological characteristics of the desert *Nostoc* were compared with the species of *Nostoc* described in the literature. Although our strains were similar to previously described species such as *N. punctiforme*, *N. paludosum*, *N. commune*, *N. microscopicum* and *N. muscorum*, they did not fit any of these taxa well when all characters (including ecological preference) were used. The 16S rRNA gene sequences of the strains CM1-VF10, CM1-VF14, CNP-AK1 and JT2-VF2 were aligned with published *Nostoc* sequences from GenBank and other desert *Nostoc* sequenced at John Carroll University. *Pseudanabaena*, *Cylindrospermum* sp. and *Anabaena spiroides* from GenBank were used as outgroups. In addition, the ITS regions of all four desert taxa, a typical *Nostoc commune* from European temperate soils, *Nostoc punctiforme* PCC73102 from cycad roots were compared by examining secondary structure. The combined analysis provided compelling evidence that CM1VF10, CM1VF14, and JT2VF2 were species new to science. While CNP-AK1 (the phycobiont of the desert soil lichen, *Collema tenax*) had some significant discontinuities from its closest relative, *N. punctiforme*, we

consider the evidence currently insufficient to separate these two strains. The purported phycobiont of this lichen is *N. commune*, and we have demonstrated that this is incorrect. One of the desert strains (JT2-VF2) likely represents a new genus. Morphological autapomorphies, distinctive SSU rRNA gene sequence, and differences in secondary structure of the 16S-23S ITS provide congruent evidence for this conclusion.

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**INITIAL CHARACTERIZATION OF THE TERMINAL DOMAINS OF LINEAR PLASMID-LIKE DNAs FROM ERNODESMIS VERTICILLATA (CHLOROPHYTA)**

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Terminal regions of linear plasmid-like DNAs from the chloroplasts of *Ernodesmis verticillata* (Kützinger) Børgesen were characterized. The 2.2-kb molecules are believed to consist of a single strand of DNA folded back on itself in a hairpin configuration. The 5' end of isolated molecules is capable of being phosphorylated with polynucleotide kinase, and the 3' end can be homopolymeric tailed with terminal deoxynucleotidyl transferase. Thus, both ends are accessible for further manipulation. Exonuclease III and lambda exonuclease digest native molecules only after heat denaturation and certain cooling regimes, suggesting that conformational changes brought about by de- and renaturation make the 3' and 5' ends (respectively) vulnerable to exonuclease activity. The cloning strategy involved poly-G tailing of native molecules, and hybridizing them to a poly-C-tailed vector. Two rounds of cloning the 3' ends yielded a total of 14 clones. Molecular sequencing revealed that two distinct clones have extensive terminal inverted repeats (TIRs). Short TIRs are also evident in most clones, possibly indicating that molecular foldbacks protect the 3' ends. Multiple tandem and direct repeats are also prevalent near the 3' termini. Despite overall sequence heterogeneity among most clones, a consensus sequence found at the 5' end of these clones probably has structural and/or functional significance. Pairwise sequence comparisons and the presence of extensive repeats suggest that these molecules may be highly recombinant.

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**MAINTENANCE OF GENETIC DIVERSITY IN HETEROSIGMA AKASHIWO BY TEMPORAL VARIABILITY IN ITS ENVIRONMENT**

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*Heterosigma akashiwo* (Hada) Hada ex Y. Hara et Chihara (Chromophyta: Raphidophyceae) is a coastal HAB forming species that is not known to undergo sexual reproduction. Ribosomal ITS sequences for 36 strains of *Heterosigma* have been determined and found to be identical except for two strains that had one single nucleotide polymorphism each. Despite the apparent absence of meiosis and the ostensible homogeneity of genotypes (at least at the ITS locus), different strains of *Heterosigma* display a very wide range of phenotypes. This inconsistency suggests that greater genetic diversity exists in *Heterosigma* than is evident through sequence analysis of the ITS region. Data will be presented summarizing efforts to develop better genetic markers for examining intrapopulational diversity. Results of experiments examining the effects of nutrient concentration in batch culture on growth response have demonstrated that sufficient haplotype variability exists in established culture strains to allow Darwinian selection, even when these strains are "clonal". We propose that batch culture imposes a sequence of different selection pressures on a rapidly dividing population of cells, and this environmental variation promotes the maintenance of genetic diversity. Extrapolating the batch culture scenario to the much more highly dynamic marine coastal environment, it becomes apparent that the observed phenotypic variation between *Heterosigma* strains is largely a function of a diverse haplotype distribution, maintained, in a large part, by patchy resource availability on both temporal and spatial scales.

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#### PHYLOGENETIC ANALYSIS OF THE CAULERPALES (ULVOPHYCEAE, CHLOROPHYTA) BASED ON RBCL GENE SEQUENCES

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Our research seeks to clarify the phylogeny of the Caulerpales through analyses of *rbcL* (large subunit of ribulose 1,5 biphosphate carboxylase/oxygenase) gene sequences. In a review of caulerpalean taxonomy, Hillis-Colinvaux (1984) recognized two suborders (Bryopsidineae and Halimedineae) on the basis of anatomical, physiological, and habitat characteristics. The Bryopsidineae (including the genera *Bryopsis*, *Derbesia*, and *Codium*) have cosmopolitan distributions, non-holocarpic reproduction, and homoplasty, while the Halimedineae (including *Caulerpa*, *Halimeda*, and *Udotea*) have tropical to subtropical distributions, holocarpic reproduction, and heteroplasty. Previous phylogenetic analyses based on 18S rRNA sequence data supported the hypothesis of two monophyletic suborders within the Caulerpales (Zechman et al 1990). However, cladistic analyses of morphological characters (Vroom 1998)

suggested that only the Halimedineae was monophyletic. Preliminary maximum likelihood and Bayesian analyses suggest the Halimedineae and Bryopsidineae form separate monophyletic groups, with robust support (bootstrap and posterior probabilities) for the former and moderate to poor support for the latter. The families of the Halimedineae (Caulerpaceae, Udoteaceae) form monophyletic sister groups with robust support. The freshwater family Dichotomosiphonaceae was inferred to be basal to the marine Halimedineae clade. The families within the Bryopsidineae (Derbesiaceae, Bryopsidaceae, Codiaceae) each form distinct monophyletic groups. The Codiaceae forms a basal monophyletic group to the sister clade of Bryopsidaceae and Derbesiaceae. This research was partially supported from a NSF grant (DEB-0128977 to FWZ).

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#### PROGRESS IN CHARACTERIZING THE SYMBIODINIUM SP. IN SORITID FORAMINIFERA

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Various methodological approaches are being used to characterize *Symbiodinium* spp isolated from *Marginopora vertebralis* and *Amphisorus hemprichii* from the Indo-pacific. While most of the cells in culture are non-motile vegetative cells, dinospores occur in batch cultures. We find consistent variance among the isolates in the duration and time of appearance. Nitrate (0.146 mM) supports higher populations of the isolates than does NH<sub>4</sub> (0.146 mM). Higher concentrations of NH<sub>4</sub> inhibit growth. Although Fensome et al (1993) characterized the Order Suesiales (and Family Symbiodiniaceae) as gymnodiniophycideans in which the amphiesmal vesicles are arranged in 7–10 longitudinal series we were unable to detect any plates on the surfaces of our isolates in the SEM. Following Klut et al (1988) we unsuccessfully attempted to reveal plates by using freeze fracture. We were more successful in using various chemical agents (e.g. H<sub>2</sub>O<sub>2</sub>, HOCl-) and enzymes (cellulase, chitinase) to remove surface layers. Amphiesmal plates are present but they are not polygonal or arranged in the patterns anticipated for the Symbiodiniaceae. Polyclonal antibody studies are in agreement with molecular systematic studies in the sense that there is a common relatedness; the foram endosymbiotic dinoflagellates share some common surface antigens with each other and with the one invertebrate isolate we have tested. Studies of the foraminiferal symbionts' plastids revealed considerable morphological diversity. These studies were supported by NIH/NIGMS 08168-22 and PSC-CUNY grant 64234-00 33.



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**AN OBLIGATE(?) HETEROKONT BIFLAGELLATE PARASITE IN *CODIUM FRAGILE***Lee, T. F.<sup>1</sup> & Kugrens, P.<sup>2</sup><sup>1</sup>*St. Anselm College, Manchester, NH;* <sup>2</sup>*Colorado State University, Fort Collins, CO*

Specimens of *Codium fragile* (Suringar) Hariot ssp. *tomentosoides* were collected from 9 sites in New England, and Long Island, New York at intervals throughout the years 1999-2003. Segments were removed from the thalli and chopped into fine fragments, mostly individual utricles and medullary filaments. Fragments were incubated in enriched seawater in dim light at 15C, 12:12 LD. Within 2-3 days, in almost all cases (more than 300) motile cells formed in many of the utricles and filaments. These were 10-15 micrometer elongated biflagellate heterokont cells. They appeared to consume the chloroplasts, and within 24 hours were reduced to colorless cells, about 5 micrometers long. These cells are unable to grow in *Codium* chloroplast suspensions. They appear to be always associated with *Codium* thalli, despite attempts to clean the thalli, and were never seen in utricles or filaments of intact plants. Their ultrastructure is under investigation and will be reported on here.

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**DEVELOPMENT OF MACROALGAL (SEAWEED) TAXONOMIC KEYS UTILIZING DIGITAL & MEDIA TECHNOLOGY**

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Digital, user-friendly seaweed identification keys and instructional materials can provide information that allows students and researchers to enhance and improve marine field and ecological studies by including macroalgae. In much of the scientific literature, the seaweeds are only characterized to division (red, green, and brown), sometimes to genus, and very rarely to species; so there is clearly a need for a reference that facilitates the identification of the seaweeds. Many of the problems occur because of the lack of user-friendly identification keys. However, it is not necessary to be an expert to identify seaweeds as many of the key characteristics used in their identification can be ascertained with the naked eye, a hand lens and an inexpensive microscope. What is needed is an identification guide that uses and displays important characters and identification structures visually (both macroscopic and microscopic) so that research scientists, students, teachers and the general public will be able to identify the seaweeds with confidence. We are using Lucid Professional software to construct digital keys for the identification of macroalgae from the Texas Coastal Bend. The

advantages include: 1) key characters along with descriptions and images for specific macroalgae may be chosen from a geographic area of choice, 2) the user may evaluate the characters in any order and difficult or uncertain characters may be ignored, 3) descriptive pages of images, definitions, video clips and illustrations may be included for each taxon, and 4) the key can be displayed as a website or packaged as a CD to be used with laptop computers in the field.

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**RECOGNIZING CRYPTIC SPECIES: INVESTIGATIONS INTO THE MOLECULAR RELATEDNESS AND MORPHOLOGICAL DIVERSITY OF THE SPECIES OF THE RED ALGAL GENUS *PLOCAMMIUM* (PLOCAMIALES, FLORIDEOPHYCEAE)**Lehmkuhl, K. V.<sup>1</sup> & Saunders, G. W.<sup>2</sup><sup>1</sup>*Bamfield Marine Sciences Centre, Bamfield, BC;* <sup>2</sup>*Centre for Environmental and Molecular Algal Research, Department of Biology, University of New Brunswick, Fredericton, NB*

Small-subunit ribosomal DNA (SSU) sequences used in combination with anatomical and morphological data have supported elevation of the Plocamiaceae to ordinal status. The Plocamiales currently includes two genera: the free-living *Plocamium* Lamouroux; and the adelphoparasite, *Plocamiocolax* Setchell. *Plocamium* includes upwards of 40 species, which are widely distributed throughout the world's oceans. Most species are clearly defined anatomically and biogeographically. In this regard the type species, *P. cartilagineum* (L.) Dixon (type locality: reportedly Northern Europe), is an exception. *Plocamium cartilagineum* is considered cosmopolitan in distribution, with individuals varying in size, and in the number and habit of ramuli in their alternating series. We have sequenced the large-subunit ribosomal DNA (LSU) for representatives of *P. cartilagineum* from France, Ireland, Norway and Scotland as well as isolates from Australia and the North Pacific. These data indicate that many cryptic species, four within Europe itself, are currently included within the concept of *P. cartilagineum*. We present molecular, biogeographical, ecological, and morphological/anatomical data characterizing these European forms and provide taxonomic revisions that reflect this diversity within *P. cartilagineum*.

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**SENSITIVITY OF CYANOBACTERIA TO A POTENTIAL BIOLOGICAL CONTROL AGENT, BACTERIUM SG-3**Wilkinson, K.<sup>1</sup>, Walke, H. L.<sup>2</sup> & Lembi, C.<sup>1</sup><sup>1</sup>*Botany and Plant Pathology, Purdue University;* <sup>2</sup>*School of Biological Sciences, Louisiana Tech University*

Cyanobacteria cause many problems in freshwater ecosystems. For example, the production of off-flavor compounds by cyanobacteria causes serious problems in catfish aquaculture. Control of cyanobacteria is generally limited to treatment with copper compounds, which are non-selective and sometimes ineffective at controlling certain species of cyanobacteria. Biological control could provide selective management by removing unwanted species while leaving desirable algae species. A bacterium (SG-3) (NRRL B-30043) lyses a number of planktonic species of cyanobacteria including bloom-forming species of *Anabaena* and *Oscillatoria*. We tested SG-3 for activity against 10 isolates, representing seven species, of mat-forming cyanobacteria within the genera *Oscillatoria*, *Lyngbya*, and *Phormidium*. Plugs (0.5 cm diameter) were cut from mats of the cyanobacterium, inoculated with liquid cultures of SG-3, and incubated as static cultures. The reduction in dry weights ranged from -0.5% to 90% compared to the untreated controls and appeared to be species specific. For example, dry weight reductions of *Oscillatoria* deflexoides and *O. amoena* ranged from 80 to 90% whereas the reduction of *O. limosa* tended to be lower at 36 to 72%. Although results varied among and within species, they indicate that this bacterium could have potential for use as a biological control for mat-forming cyanobacteria. Light microscopic observations indicate the bacteria do not penetrate the cyanobacteria cells. Currently, we are studying the possible causes of the observed cell lysis.

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#### **PHYLODIVERSITY OF GREEN ALGAE (CHLOROPHYTA) FROM DESERT MICROBIOTIC CRUSTS**

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Deserts are not thought of as biodiversity hotspots, but desert microbiotic crust communities represent a largely unknown community type rich in diversity of eukaryotic and prokaryotic taxa. These ecologically important communities have received much attention because of their role in nutrient cycling and soil stabilization in deserts, but they defy characterization by the traditional approach to assessing biodiversity by counting species. While genetically diverse, taxa characteristic of desert crusts are difficult to identify to the species level due to convergent evolution toward simple morphologies, phenotypic plasticity, or poor knowledge about particular lineages. Focusing on the green algae, we show that while biodiversity is difficult to measure in these communities, phylo diversity provides a surrogate measure that more accurately portrays the diversity of organisms, and one that is standardized across the variety of life histories, reproductive strategies and morphological variability that creates problems with species-count-

ing measures. Bayesian phylogenetic inference uses MCMC simulation to generate phylogenies sampled in proportion to their Bayesian posterior probability. The length of a segment in any of these trees corresponds to the amount of change in the lineage, measured as the expected number of substitutions/nucleotide site. Comparisons of segment lengths corresponding to desert vs. other green algal lineages provides a means of addressing questions of relative genetic diversity, or phylo diversity, without complications arising from the difficulty of counting species. Our data illustrate the impact of desert green algae to overall knowledge of the green algal phylogenetic tree.

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#### **TEACHING BIOLOGICAL RESEARCH USING BROWN ALGAE**

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Mentoring students in investigative research is a key part of actively engaging them in learning biology. While this is best accomplished by involving students in individualized research projects, it is not always feasible to offer such an opportunity to all students. A course in Biological Research that focuses on doing research with a particular "model system" has been an indispensable part of our biology curriculum. Algae provide ideal model systems for this purpose, and kelp gametophytes, including *Macrocystis* and other genera, have provided excellent results. In the first half of this course, students learn to access information in the published literature, while writing a literature review that relates to a research project that they will propose and design together with a student partner. Simultaneously, they learn useful techniques to grow and manipulate kelp gametophytes. During the second half, they mainly conduct the proposed research. Students present their research proposals and final results to the rest of the class. Most research projects dealt with growth responses or molecular genetics. Growth and survival of gametophytes was determined in response to nutrients or toxicants. Kelp DNA was amplified by PCR in a variety of projects, including later analysis by sequencing or SSCP and RAPDs. Kelp gametophytes proved to be an ideal organism for this course, since growth responses can be measured relatively rapidly, and DNA extraction is relatively simple due to their filamentous morphology.

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#### **EFFECTS OF CHELATED IRON ON GAMETOGENESIS IN KELP GAMETOPHYTES**

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Gametogenesis in several species of kelp gametophytes was investigated in relation to the supply of chelated iron (Fe-EDTA) in the culture medium. Spores of *Laminaria setchellii*, *Egregia menziesii*, *Alaria marginata*, *Dictyonium californicum*, and *Macrocystis pyrifera* were grown in Provasoli's Enriched Seawater with and without added Fe-EDTA. All species showed a decrease in gametogenesis without added Fe-EDTA. Gametophytes of *E. menziesii* showed predominant gametogenesis even without supplied iron, resulting in all cells being converted to gametes so that vegetative growth did not continue. Vegetative gametophytes were obtained in the other species. *M. pyrifera* did not show any gametogenesis without added Fe-EDTA. *L. setchellii*, *A. marginata* and *D. californicum* were intermediate in their response. In addition, Fe-EDTA supply was delayed by 1, 2 and 3 weeks with spores of *M. pyrifera*, resulting in an intermediate degree of gametogenesis which decreased as the delay grew longer. Fe-EDTA supply was also investigated using isolated female gametophytes of two strains of *M. pyrifera* and one of *Macrocystis integrifolia*. None of the three strains produced gametes without the addition of Fe-EDTA. Gametophytes of *M. integrifolia* required the least amount of added Fe-EDTA to achieve gametogenesis while gametophytes of *M. pyrifera* required higher amounts, with the two strains showing different responses. Thus, by varying the supply of chelated iron, it is possible to control vegetative growth or gametogenesis in most of these kelp species. In addition, different species and individuals show varied responses to added chelated iron.

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#### **EFFECT OF KARLODINIUM MICRUM TOXIN ON SUCCESS OF THE PARASITIC DINOFLAGELLATE AMOEBOPHYRA SP.**

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Parasitic dinoflagellates of the genus *Amoebophrya* infect and kill bloom-forming dinoflagellates, including the toxic species *Karlodinium micrum*. Unlike non-toxic hosts, *K. micrum* is partially resistant to infection, a trait that may be related to toxin production. Here we tested the hypothesis that parasitism of *K. micrum* is inversely related to toxin concentration in the culture medium. Time-course studies were conducted to determine the influence of extracted toxin and toxin carrier (methanol) on host growth, parasite prevalence, and parasite load. Results indicate that methanol concentrations below 0.1% have no effect on these variables. When methanol concentration was maintained below 0.1%, extracted toxin equivalent to

100 to 10,000 *K. micrum* per ml had no effect on host abundance. We are currently analyzing sample to assess the fate of *Amoebophrya* dinospores when exposed to *K. micrum* toxin. We will also consider the effect of intracellular host toxin on parasite success, by examining the fate of *Amoebophrya* dinospores when inoculated to *K. micrum* cultures that express different levels of toxin production. Understanding the effect of toxins on parasite success will contribute to our knowledge of host-parasite biology and provide insight into the role of dinoflagellate toxins as a defense against parasitism.

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#### **CHARACTERIZATION OF AN ARSENATE REDUCTASE ENCODED BY ORF slr0946 FROM THE CYANOBACTERIUM SYNECHOCYSTIS SP. PCC6803**

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Arsenic is a human carcinogen that is frequently encountered in high concentrations in both aqueous environments and in human drinking water supplies. Many microorganisms have evolved arsenical detoxification systems in which arsenate (AsV) is enzymatically reduced to arsenite (AsIII). In this study we report the expression and characterization of the first arsenate reductase from a cyanobacterium, the product of slr0946 from the cyanobacterium *Synechocystis* sp. PCC6803. Slr0946 was expressed as an C-terminally 'His-tagged' fusion protein. The recombinant protein efficiently catalyzed the reduction of arsenate to arsenite using reducing equivalents supplied from NADPH via glutathione and glutaredoxin. Thioredoxin, the traditional reductant for previously characterized ArsC's, was ineffective. Slr0946 displayed both relatively high catalytic efficiency,  $V_{max}$  3.1  $\mu\text{mol}/\text{min}/\text{mg}$ , as compared to other arsenate reductases. Its  $K_m$  for arsenate was 1.25 mM. Slr0946 also exhibited vestigial phosphohydrolase activity toward p-nitrophenyl phosphate. Both arsenate reductase activity and phosphohydrolase activity were dependent upon the presence of the conserved cysteine residue, Cys8. Mutation of either of two closely paired cysteines in Slr0946, Cys80 or Cys82, but not Cys13 to serine eliminated arsenate reductase activity. However, each of these altered forms did catalyze the hydrolysis of pNPP, indicating that these cysteines were not essential to this catalytic function. It has further been suggested that glutathione forms a mixed disulfide with one of these cysteines during catalysis. This suggestion was at least partially supported by the demonstration that Slr0946 forms an SDS-resistant complex with [<sup>35</sup>S]glutathione in a manner that is dependent on the presence of both Cys8 and arsenate.

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### ASSESSING MICROALGAL MORPHOLOGY FROM CENTURY-OLD HERBARIUM SHEETS USING SEM

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Traditionally, type specimens have not been deposited for most unicellular algae. There are few known holotypes and those that are available are often of limited use. Samples from taxa described before 1900 commonly consist of materials dried down on herbarium sheets or mica. Dried algal samples can be rehydrated, but preservation is very poor and little structure can be determined from them. Whether wet or dry, the amount of information that can be derived from such samples using conventional light microscopy is limited. However, identification of microscopic algae from herbarium samples may be necessary when the original descriptions and/or illustration(s) are inadequate, questionable or when a taxon is under revision. We were able to derive high-resolution images from dried algal samples using a JEOL 6300 field emission SEM. For example, we have imaged samples of *Euglena spirogyra* that were dried on mica chips in October of 1899 and stored in the Michigan State University herbarium. The overall shape of the cell body and the arrangement and ornamentation of the pellicle strips were clearly visible. Because of this success we decided to examine a variety of algal samples that had been preserved on mica. Algae such as *Staurastrum*, *Scenedesmus*, or *Mallomonas* (particularly the scales), retained the most structure while others such as *Pediastrum* and *Pandorina*, retained sufficient structure to allow their identification. We suggest that FESEM should be more broadly used for assessing structure in dried herbarium samples.

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### IDENTIFICATION OF *PFIESTERIA PISCICIDA* (DINOPHYCEAE) AND *PFIESTERIA*-LIKE ORGANISMS USING ITS-SPECIFIC PCR ASSAYS

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The putative harmful algal bloom dinoflagellate, *Pfiesteria piscicida*, frequently co-occurs with other morphologically similar species collectively known as *Pfiesteria*-like organisms (PLOs). This study specifically evaluated whether unique sequences in the ribosomal internal transcribed spacer regions (ITS1 and ITS2) could be used to develop PCR assays capable of detecting PLOs in natural assemblages. ITS regions were selected because they are more variable than the flanking small subunit (SSU) or large subunit (LSU) ribosomal RNA genes and more likely to contain species-specific sequences. Sequencing of the ITS regions revealed unique oligonucleotide primer binding sites for *Pfiesteria piscicida*, *Pfiesteria shumwayae*, Florida "Lucy" species, two cryptoperidiniopsoid species, "H/V14" and "PLO21," and the estuarine mixotroph, *Karlodinium micrum*. These PCR assays had a minimum sensitivity of 100 cells in a 100 mL sample (1 cell mL<sup>-1</sup>) and were successfully used to detect PLOs in the St. Johns River system in Florida, USA.

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### ASTACENE IS A MAJOR SECONDARY CAROTENOID IN THE GREEN ALGA *PALMELLOCOCUS MINIATUS*

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Astacene (3,3'-dihydroxy-2,3,2',3'-tetrahydro-b,b'-carotene-b,b-dione) is a red carotenoid reported in small quantities in some green algae, higher plants, and animals. However, it has been generally believed that astacene is an oxidative artifact of astaxanthin (3,3'-dihydroxy-b,b-carotene-4,4'-dione). Astaxanthin is a naturally occurring pigment and a structural analogue of astacene. A recently isolated unicellular green alga, *Palmellococcus miniatus*, has been found to synthesize and accumulate large quantities of red secondary carotenoids under certain environmental stress conditions. In this study, chromatographic, spectrophotometric and mass spectrometric evidence has been used to confirm that astacene is a natural occurring pigment, which can comprise over 70% of the secondary carotenoids in stressed cells. Electron microscopy revealed that the accumulation of astacene was accompanied by the formation of numerous lipid bodies outside of the chloroplasts. Lipid bodies were isolated from the cells by French pressure cell disruption, followed by sucrose gradient density centrifugation. HPLC analysis indicated that lipid bodies were the sites of astacene accumulation. The possible physiological roles and distribution of this pigment among several groups of green algae will be discussed.

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**NITROGEN FIXATION BEHAVIOR OF A TERRESTRIAL HETEROCYSTOUS CYANOBACTERIUM, *NOSTOC FLAGELLIFORME*, AS AFFECTED BY AVAILABLE NUTRIENTS IN LIQUID SUSPENSION CULTURE**

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*Nostoc flagelliforme* is an edible terrestrial cyanobacterium of great economic value which is often distributed in arid or semi-arid steppes in many parts of the world. The relationship between N<sub>2</sub>-fixing capacity and nutrient availability of *N. flagelliforme* under various growth conditions was investigated. Phosphorus had a profound effect on nitrogenase activity (NA) of the alga over the concentration range of 2.3 μM–0.46 mM inorganic phosphorus (Pi) in the cultures, with a maximal NA at 23 μM Pi. The optimal temperature for NA was determined by the Arrhenius equation to be approximately 25° C. However, the response of NA to different phosphorus concentrations was not affected by temperature variation. Micro-nutrients in the media also affected NA of the alga. A 2-fold strength of both B and Ca (relative to the standard BG110 medium) significantly enhanced NA. In addition, simultaneous enrichments with B, Ca, Fe and Mo exerted marked beneficial effects on NA, and such effects were presumably in an interactive and synergistic manner. In contrast, removal of Mo gave rise to a drastic decrease in growth rate, while the NA was only moderately decreased. NA in the Mo-, Co-, and Fe-free cultures was respectively inhibited, as compared to cultures devoid of Ca, Fe, Mg, Mn, Zn and Cu, respectively. Results of the present study suggested that *N. flagelliforme* could be an important contributor of nitrogen to the terrestrial ecosystem because of its strong nitrogen-fixing ability even in an infertile environment.

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**EVOLUTIONARY RELATIONSHIPS IN THE FAMILY TRENTEPOHLLIACEAE (CHLOROPHYTA; ULVOPHYCEAE)**

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The subaerial green algal family Trentepohliaceae is an assemblage of commonly overlooked algae well represented in warmer climates. Our ongoing phylogenetic research to elucidate the evolutionary relationships within this family strongly supports its monophyly. We are currently evaluating the evolutionary relationships among and within the main genera: *Trentepohlia*, *Phycopeltis*, *Cephaleuros* and *Stomatochroom*, and are using the nuclear-encoded 18SSU rDNA as well as newly designed chloroplast-encoded *rbcL* primers. The current generic concept in the Trentepohliaceae is challenged by the molecular analysis. Morphological features such as the solitary vs. grouped sporangiate lateral, and isomorphic vs. heteromorphic alternation of generations are emerging as important phylogenetic markers in the family. Free filaments vs. coalesced filaments (disc-shaped thallus) are morphological characters scattered among the lineages. The genus *Cephaleuros* is a well supported monophyletic genus. One of the most common species in the literature is *Cephaleuros virescens* Kunze in Fries, which is reported to have a worldwide distribution in tropical and subtropical terrestrial ecosystems. Analysis of DNA sequences reveals that this "species" may encompass a number of taxa.

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**MARINE ALGAE FROM THE OIL/GAS PLATFORMS IN THE NORTHWESTERN GULF OF MEXICO**

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The macroalgal diversity of the oil and gas platforms in the northwestern Gulf of Mexico is being studied. These artificial habitats provide physical support for a significant community of marine organisms. During a cruise in May 2002 samples were collected from four platforms off the Louisiana coast selected to follow a horizontal gradient that will allow comparison of species diversity, relative abundance, and vertical and horizontal distribution of near-shore and outer-shelf areas. A zonation of algal communities in the intertidal was visible at all sites consisting of barnacles and small blades of green algae, mainly *Ulva fasciata* and several species of *Enteromorpha*. The ongoing

analysis of the samples has resulted in 24 taxa: Chlorophyta 7, Rhodophyta 12, Phaeophyceae 2, and Cyanobacteria 3. Seven new records have been added to the oil/gas platform flora of Louisiana. The total number of algal taxa recorded in Louisiana from these artificial reefs is ca. 100 (excluding Cyanobacteria), with 20 percent found only on this steel archipelago and not on other natural substrates on the Louisiana coast. A horizontal and vertical distribution of algae appears to relate to environmental factors, possibly those associated with the run-off of the Mississippi river. A digital data bank of algal images for the project can be found at <http://chapmanlab.lsu.edu/digitalalgae>. This website also has an extensive list of literature on medical and biotechnological uses of algae and products. This project was supported in part by the Minerals Management Service

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#### THE PHYLLOPHORACEAE (GIGARTINALES, RHODOPHYTA): GENERIC CONCEPTS

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The Phylloporaceae stands out in exhibiting a wide range of life history types making it unusually interesting to assess the phylogenetic importance of reproductive traits relative to classification criteria. Type of life history and position of the reproductive structures on the thallus have traditionally formed the basis for separating the genera in the family; however, DNA sequence analyses of *rbcL*, LSU rDNA and ITS instead indicate a lack of correlation between type of life history and phylogenetic relationships among the established taxa. We will present a revised generic classification of the family that downplays life history features and highlights the different ontogenetic pathways leading to the establishment of the fruiting bodies. The study will answer the question which morphological features can be used as meaningful indicators of relationships in the Phylloporaceae. The results will be addressed in light of global biogeographic hypotheses for the family.

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#### IS THE FRESHWATER DIATOM FLORA OF HAWAI'I DEPAUPERATE?

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The relatively low number of freshwater diatom taxa reported from Hawai'i has produced biogeographic speculation. Before examining ecologic or biogeographic explanations, it seemed reasonable to test a sampling hypothesis given the history of diatom

collecting in this remote archipelago. Prior to the publications of Rushforth and his associates in the 1980's, relatively few diatom samples had been collected. And very few diatom taxa were reported to be endemic. Even after their publications, these numbers remained low. Because earlier samples were collected from a few low elevation streams and ponds (with two exceptions), the present study extended sampling to higher elevations, increased the number of drainages sampled, and sampled at several seasons. Less than half the samples have been thoroughly studied, but from 76 samples 230 new reports were found with four new taxa published and with many more taxa to be described. Most of these new reports are taxa rare in abundance or found in remote sites. It now appears that the known diatom taxa list for the Hawaiian Islands is an artifact of sampling effort.

### 111

#### EFFECT OF HERBICIDE EXPOSURE ON GROWTH AND PHOTOSYNTHETIC PERFORMANCE IN HALOTOLERANT ALGAE

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Recent studies indicate that herbicide contamination of riverine and nearshore environments could be affecting the competitive fitness of algae and aquatic vegetation, thereby, altering community structure and ecosystem health. This study is the first in a series designed to investigate the effects of herbicide exposure on growth and photosynthesis in two halotolerant species of green algae (*Dunaliella* sp. and *Nannochloris* sp.), isolated from the Great Salt Plains (GSP), OK. The GSP is the remnant of an ancient ocean, encompassing ~ 65 km<sup>2</sup> of variably hypersaline flat land, fed by tributaries of the Arkansas River. As water flows over the flats, it brings with it, not only increased amounts sediment and nutrients, but also herbicides that are commonly applied to surrounding agricultural lands. Since salinity stress and herbicide exposure trigger similar cellular responses (i.e., increase in anti-oxidant pigment content, down-regulation of photosynthesis, up-regulation of respiration and ATP synthesis), acclimation to high salinity might confer tolerance to herbicide exposure in salt-tolerant algae. To investigate this hypothesis, experimental cultures are being acclimated to salinities of 5 and 15 ppt and subsequently exposed to one of four herbicide concentrations (0, 1.0, 10.0 or 100 uM of DCMU, atrazine or hexazinone). Growth, oxygen evolution, fluorescence and pigment content data are being gathered to assess the effects of salinity and herbicide exposure on the overall performance of *Dunaliella* and *Nannochloris*. The main objective of this research is to determine to what extent halotolerant algae are

able to tolerate exposure to commonly applied agricultural herbicides.

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### MAKING BAC LIBRARIES FROM ALGAE, NON-SEED AND SEED PLANTS: FUNDAMENTAL PUBLIC RESOURCES FOR PLANT SCIENCE

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The evolutionary transition to life on land and the diversification that followed required solving a series of problems such exposure to air and solar irradiation, life in a desiccating environment, etc. Plants solved these challenges with biochemical, cellular, anatomical and morphological innovations. We will provide access to the genetic basis of most of these innovations by making high-quality bacterial artificial chromosome, or BAC, libraries of 16 green plant species which are key in the evolutionary transition to land. These taxa include: *Volvox carteri*, *Caulerpa mexicana*, *Mesostigma viride*, *Coleochaete orbicularis*, *Chara* sp., *Marchantia polymorpha*, *Anthoceros* sp., *Lycopodium lucidulum*, *Angiopteris evecta*, *Ceratopteris richardii*, *Marsilea quadrifolia*, *Amborella trichopoda*, *Nuphar adventa*, *Acorus gramineus*, *Liriodendron tulipifera*, *Mimulus guttatus*. All libraries will be made available to the public for a minimal cost via Clemson University Genome Institute and Arizona Genomics Institute as soon as they are made and characterized. Key innovations and our current progress, including the timing of the planned availability of these 16 BAC libraries to the public at large, will be presented. Funded by the NSF's First One Hundred Program, 2002–2005, IBN #0208502 (lead institution), #020211611, #0207110, and #0207202.

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### TOWARD RESOLUTION OF THE FUZZY NODES IN GREEN PLANT PHYLOGENY

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We are funded to resolve the primary pattern of evolutionary diversification among green plants, and to establish a model for doing so that will be applicable to other groups of organisms with long evolutionary histories. To achieve this goal we will 1) complete a matrix of whole genome sequences for chloroplast and mitochondria and develop either nuclear or organellar bacterial artificial chromosome, BAC, libraries for about 50 representatives of the critical deep-branching lineages of green plants; 2) produce a comprehensive set of comparable morphological and ultrastructural data for these same taxa; 3) incorporate inferences from across the phylogenetic hierarchy in green plants using methods designed to permit scaling across studies. We shall indicate how this work will link to other research being conducted on green plants and various scales, especially the concatenation of our data sets with theirs. We will present the fuzzy nodes we have chosen to resolve and discuss our choices of taxa in this preliminary report. Funded by NSF's Tree of Life Program, 2002–2006, DEB #0228655 (lead institution), #228432, #0228679, #0228729, #0228660, #0228576.

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### VARIABLE MORPHOLOGY AND BIOMECHANICAL PERFORMANCE OF GENICULA IN CALLIARTHON TUBERCULOSUM

Martone, P. T.

Genicula of the articulate coralline *Calliarthron tuberculatum* (Postels & Ruprecht) Dawson are composed of a single tier of medullary cells that decalcify endogenously to form flexible joints. Uncalcified, unpigmented genicular cells lose their cytoplasm and organelles over time and are often presumed to be inactive. Nevertheless, as the flexible components in an otherwise rigid system, genicula probably play a critical role in the mechanical stability of *C. tuberculatum* in the exposed, low-intertidal habitat. I investigated how the size and tensile strength of genicula vary

along individual thalli. I found that the force (N) required to break a specific geniculum is closely correlated to the amount of algal surface area (mm<sup>2</sup>) distal to it. That is, a geniculum proximal to a large frond, which would experience a high drag force in the field, endured a greater breaking force than a geniculum closer to the tip. This suggests that, to avoid breakage, plants may adjust the size or composition of genicula to mechanically compensate for distal growth and increased drag. I will discuss how material properties and cross-sectional area may influence performance.

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#### **DO ALGICIDAL BACTERIA SIGNIFICANTLY INFLUENCE THE DYNAMICS AND THE DEMISE OF MARINE ALGAE BLOOMS?**

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It has now been well established that bacteria perform key ecosystem functions in the marine environment, mainly as utilizers of organic matter that is ultimately derived from phytoplankton. Over the past decade, many reports have revealed the existence of bacteria capable of killing live phytoplankton cells, coining the term? algicidal bacteria?. Further, these algicidal bacteria increase in abundance concurrent with the decline of algal blooms, suggesting that they may somehow affect algal bloom dynamics. We will synthesize the existing knowledge of algicidal bacteria interactions with marine eukaryotic microalgae. We will critically evaluate the effectiveness of the current methods to characterize the algicidal phenotype in an ecosystem context. We will discuss the literature on the phylogenetic identification and affiliations of algicidal bacteria, specificity of algal-bacterial interactions, characterization of algicidal molecules, and enumeration of algicidal bacteria during algal blooms. We conclude that, due to the current methodological limitations, the evidence for algicidal bacteria causing algal bloom decline is circumstantial. New methods, including culture-independent methods, are needed to test hypotheses on the significance of algicidal bacteria in algal bloom dynamics and termination.

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#### **DEMOGRAPHY OF *ALARIA MARGINATA* (POST. & RUPR.) ALONG THE BIG SUR COAST OF CALIFORNIA**

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Large intertidal kelps are a common feature of wave-exposed temperate reefs, yet knowledge of their life histories and persistence in such environments is based largely on a few studies of *Postelsia palmaeformis* (Rupr.) in the Pacific Northwest. Surveys showed that the large (to >5 m long) kelp, *Alaria marginata*, dominates much of the exposed mid to low rocky intertidal shore along the Big Sur coast of California, at densities of up to 500/m<sup>2</sup>. Experimental clearings and controls were established in July 2001 at two sites to assess timing of recruitment and plant survivorship. Plants were collected monthly to determine population growth and reproduction. Results show that the plant is an annual. Sporophyte recruitment occurred primarily on geniculate corallines and residual *Alaria* holdfasts in early spring. Maximum growth rates were estimated at 1.6 m/mo in April and averaged 0.67 m/mo over the entire growing season (February–July). Sorus development began as early as April, peaked in late summer/early fall and decreased as adult plants were tattered or ripped from substrate by winter surf. However, spore release was generally minimal except between October and January and appeared to be stimulated by high water motion. Survivorship of plants beyond one year was rare (<1% in natural stands). The persistence of this annual, intertidal kelp appears to be related to precise timing of sporophyte recruitment, growth, and reproduction, and the presence of substrate that presumably enhances spore settlement, and/or gametophyte-young sporophyte survivorship.

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#### **OREGON'S FIRST PHYCOLOGISTS? LEWIS AND CLARK DISCOVER THE ALGAE**

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This year (2003) marks the 200th anniversary of beginning of the epic expedition of Lewis and Clark to explore western North America. In addition to searching President Thomas Jefferson, they mapped the region and explored its flora and fauna. Meriwether Lewis was an experienced amateur field botanist, but Jefferson sent him to Philadelphia for training with Benjamin Smith Barton, the foremost American botanist of the era. Lewis learned well and collected hundreds of botanical specimens, over 200 of which survive and are housed at the Academy of Natural Sciences in Philadelphia. Scarcely noticed among the many vascular plants collected is an alga from the mouth of the Columbia River, which was assigned initially to the genus *Fucus* (as were most brown algae of that era) but is the seaweed *Egregia menziesii* (Turn.) Aresch. The specimen of *Egregia* in the Lewis and Clark Herbarium bears a number of epiphytic filamentous algae, which will be illustrated in this talk. Lewis and Clark record this seaweed in



their journals and also describe one or possibly two other large kelps (*Nereocystis luetkeana* (Mert.) Post. & Rupr. in picturesque language. The journals do not mention freshwater algae in any detail, although a mineral spring feeding into the Missouri River near Great Falls Montana, which played an important role in treatment of their Shoshone guide Sacagawea, is currently dominated by *Chara*. This lecture will present a summary of the botanical legacy of the Lewis and Clark expedition, and especially its phycological findings, which remain largely unknown.

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**A NUTRITIONAL ANALYSIS OF THE TURF ALGAL DIET AND FECAL PELLETS OF THE GREEN TURTLE, *CHELONIA MYDAS* L**

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In 1978, the green turtle, *Chelonia mydas*, was listed as threatened in the United States under the Endangered Species Act. Any knowledge gained from an understanding of the diet and how it affects this species' ability to survive is crucial. Turf algae, the primary component of the diet of *Chelonia mydas*, and turtle fecal pellets were collected from Kaloko-Honokohau National Historical Park on the island of Hawaii at monthly intervals. The turf algae and fecal pellets were subjected to nutritional analyses for protein, carbohydrate, lipid, ash, and caloric content. The fecal pellets were higher in protein content than the turf algae, which may be related to fermentation carried out by bacteria in the turtle hindgut that increases the amount of protein available for absorption. From the nutritional data, assimilation efficiencies were calculated for the green turtle.

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**MARINE ALGAE OF POHNPEI AND ANT ATOLL WITH COMPARISONS TO OTHER PACIFIC ATOLLS AND ISLAND GROUPS**

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A study of benthic marine algae collected from Pohnpei Island and Ant Atoll, Federated States of Micronesia, documented the occurrence of 113

species of red algae (Rhodophyta), 60 species of green algae (Chlorophyta) and 17 species of brown algae (Phaeophyta), many of which are new records for Pohnpei, Ant Atoll and Micronesia. Added to reports from the literature, a total known marine benthic flora of Ant and Pohnpei (exclusive of Cyanobacteria and crustose coralline algae) now stands at 241. The high island, volcanic nature, and larger size of Pohnpei which is very different from the low, calcareous geomorphology of smaller Ant Atoll, led us to expect higher species numbers from Pohnpei relative to Ant, and greater differences in their algal floras than we found. Comparisons of the Ant-Pohnpei algal flora were also made using Jaccard and Sorensen's indices to Ifaluk Atoll (Caroline Islands), Enewetak Atoll (Marshall Islands), the Northern Mariana Islands, and the distant south Pacific island groups of Fiji and French Polynesia. Biogeographical investigation of the marine algae flora revealed that Ant Atoll and Pohnpei have a large number of widespread and Indo-Pacific species, but very few probable regional endemics.

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**EXPLORATION OF MORPHOLOGICAL VARIATION WITHIN THE GENUS *PEDIASTRUM* MEYEN 1829 (CHLOROPHYCEAE, CHLOROPHYTA)**

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Monographic works on the green algal genus, *Pediastrum* Meyen 1829 (Chlorophyceae, Chlorophyta), have described species, varieties and forms based on such characteristics as the size and shape of the marginal cells, pattern of cell wall sculpturing and extent of cell wall sculpturing. Depending on the author, the number of taxa assigned to the genus *Pediastrum* varies. Due to the lack of quantitative value to these characteristics, it has been difficult for other researchers to assign appropriate taxonomy to wild isolates. A molecular phylogeny including multiple strains from both culture collections and wild samples confirms relationships found by previous molecular studies on fewer taxa, in which the family Hydrodictyaceae forms a monophyletic group within the Sphaeropleales, and that the genera *Hydrodictyon* and *Sorastrum* are derived from *Pediastrum*. *Hydrodictyon* forms a monophyletic clade and consists of three species, *H. reticulatum*, *H. africanum*, and *H. patenaeforme*. Multiple isolates of *H. reticulatum* reveal little genetic variation between different geographic localities. Inclusion of wild isolates permits a more thorough exploration of morphological variation within the genus *Pediastrum*, and what characters may be taxonomically informative, particularly in the species *P. boryanum* and *P. duplex*. Wild isolates

sampled from different areas also offers information regarding geographic variation and potential morphological convergence.

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### **INFLUENCE OF BENTHIC VS. PELAGIC SEEDING ON COASTAL DIATOM BLOOM DEVELOPMENT**

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Diatom blooms may be initiated by cells that have survived in the plankton or germinated resting stages from the sediments. However, it is not well understood how these different inocula contribute to bloom development. We followed diatom community development in twenty-liter microcosms given different inocula. Surface sediment and phytoplankton were collected in Gullmar Fjord, Sweden. Replicate microcosms were then dosed with local sediment and/or plankton and incubated in situ in Gullmar Fjord. We also followed the concurrent development of the phytoplankton community in the fjord. Experiments run in both spring and fall 2002 showed that bloom development in the microcosms was significantly faster when seeded by planktonic cells. However, addition of sediment may have stimulated planktonic growth and also provided additional propagules. The type of inoculum used strongly influenced the diatom composition in the microcosms. Sediment additions, through germination of resting stages, resulted in communities dominated by *Detonula confervacea* and *Thalassiosira minima* in spring, and *Skeletonema costatum* in fall. Planktonic inocula resulted in blooms of *T. nordenskiöldii* and *Chaetoceros debilis* in spring, and *S. costatum* and several *Chaetoceros* spp. in fall. Microcosms dosed with both plankton and sediment showed a mixed species assemblage. Comparisons between the microcosms and fjord phytoplankton suggest an important role for benthic seeding of diatom blooms.

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### **LOCAL TO COASTAL-SCALE MACROPHYTE COMMUNITY STRUCTURE: SURPRIZING PATTERNS AND POSSIBLE MECHANISMS**

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Understanding large-scale patterns in ecological communities is a central goal of ecology, and yet, rigorous quantitative geographic data on distribution, abundance and diversity are almost totally lacking. Even in rocky intertidal habitats, our data on community structure are spatially and temporally limited, with most surveys limited to a few sites over short time periods. When linked to studies of community dynamics on similar scales, such studies should provide insights into the determinants of pattern at more relevant scales. In 1999 PISCO, the Partnership for Interdisciplinary Studies of Coastal Oceans, initiated survey programs aimed at determining patterns of community structure along the US west coast from Washington to Baja California. Sites are regularly spaced along the coast in a nested design, and were physically similar. Surveys used randomly placed quadrats in transects run parallel to shore in high, mid and low zones. Results show that, contrary to expectation, macroalgal diversity along the northern coast was higher, not lower than that along the southern coast. Possible factors associated with this unexpected pattern include along-coast variation in tidal amplitude, time of tide, thermal stress, upwelling intensity and resulting nutrient gradients, disturbance from storms or sand burial, and grazing. We review evidence relevant to these factors, and focus on the possible role of grazing, using field experiments done under differing oceanographic conditions along the Oregon coast as a model. Although short-term grazing rates can vary with oceanographic condition, we hypothesize that despite these results and those of many similar studies showing strong grazing effects on local spatial and short time scales, that bottom-up factors are stronger determinants of macroalgal community structure on larger spatial scales and longer time scales.

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### **PERMANENT FLUORESCENT NUCLEAR STAINING OF BINUCLEATE DINOFLAGELLATES**

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Typically, fluorescent microscopy of dinoflagellate nuclei is of poor resolution, due mainly to visual obstruction of the nuclei by plastids, pigment granules, and thecal plates. Moreover, the usual slide mounts using buffered glycerol are temporary, and fade after a week or so. We have developed a procedure to clear pigments from dinoflagellates, followed by fluorescent staining of the nuclei. The cells are then prepared as permanent mounts using an ultraviolet light-catalyzed resin to produce stained samples which may be kept for at least three years with little loss of fluorescence. This procedure can

also be used to prepare plastic embedded dinoflagellate cells which can then be sectioned at 1–2 nm, fluorescent stained, and permanently mounted. Suitable nuclear stains are DAPI, Hoechst 33258, ethidium bromide and acridine orange. The dinoflagellate (dinokaryotic), and endosymbiont (eukaryotic) nuclei are clearly visualized, revealing individual chromosomes in the dinoflagellate nucleus, and a highly lobed morphology of the endosymbiont nucleus.

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### EVIDENCE FOR LATERAL TRANSFER OF A IE INTRON BETWEEN FUNGAL AND RED ALGAL SMALL SUBUNIT RIBOSOMAL RNA GENES

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In a recent study of the North American biogeography of the red algae genus *Hildenbrandia*, the presence of group I introns were noted in the nuclear SSU rRNA gene of the marine species *H. rubra* (Hildenbrandiales). Group I introns in the nuclear encoded rRNAs have been previously reported in the Hildenbrandiales as well as the Bangiales. All reported introns within the red algae have been identified as belonging to the IC1 subclass and occur at two insertion sites in the nuclear small subunit rRNA (516 and 1506). However, an unclassified intron was discovered at position 989 in the nuclear SSU rRNA gene of a collection of *H. rubra* from British Columbia, Canada. We have determined that the intron is a member of the IE subclass and this is the first report of an IE intron and an intron in position 989 in the red algae. Phylogenetic analyses of the intron sequences reveal a close relationship between this group IE intron and similar ascomycete and basidiomycete fungal IE introns in the nuclear SSU rRNA genes at positions 989 and 1199. In addition, a common unique helix (structural signature) in the P13 domain of the *Hildenbrandia* intron and those of the fungi at the 989 and 1199 IE positions in the nuclear SSU rRNA gene also indicates a close relationship. Hence, this study provides evidence for a possible lateral transfer of the IE intron in position 989 between fungal and red algal nuclear SSU rRNA genes.

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### CHANGING ENVIRONMENTAL CONDITIONS AND CHANGES IN THE ABUNDANCES OF ROCKY INTERTIDAL SEAWEEDS ON SOUTHERN CALIFORNIA SHORES

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Environmental conditions have changed along the southern California coast during the past five decades. The human population has greatly expanded, and coastal habitats have become increasingly influenced by human activities. In addition, coastal waters have been subjected to shifts in ocean climate, including the regime shift of the 1970s that affected coastal plankton, fish, and sea bird populations. Changes in the abundances of intertidal seaweeds during this nearly fifty-year period are less well known. The earliest available data for tracking seaweed abundances were obtained by E. Y. Dawson in the late 1950s. During the mid-1970s, Mark Littler and colleagues performed a series of more intensive ecological studies at additional southern California sites. We re-sampled fourteen of these rocky intertidal sites between 1998 and 2002 to determine changes in seaweed abundances since the mid-1970s. All sites were sampled using line transect or plot-based methods similar to those used by earlier investigators. Our abundance data were then compared with values reported from the late 1950s through the mid-1970s for the same study sites. We found that macrophyte communities at many of our sites have become increasingly dominated by low-producing, crustose and articulated coralline algae and turf-forming seaweeds. During this same period, cover of several larger, and more productive, frondose algae declined. These shifts in seaweed abundances appear to have changed the structure and functioning of southern California intertidal communities through changes in the principal contributors to community primary productivity and reductions in the availability of habitat provided by algal canopies.

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### PORPHYRA BIRDIAE NEEFUS ET MATHIESON (BANGIALES, RHODOPHYTA): A NEW SPECIES FROM THE NORTHWEST ATLANTIC

Neefus, C. D.<sup>1</sup>, Mathieson, A. C.<sup>1,2</sup>, Klein, A. S.<sup>3</sup>, Teasdale, B.<sup>1</sup>, Bray, T.<sup>1</sup>, & Yarish, C.<sup>4</sup>

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Recent studies combining biochemical, molecular, and traditional morphological and ecological traits have shown that some currently recognized species of the red algal genus *Porphyra* are actually "form species" or "complexes" comprising several morphologically similar but genetically distinct taxa. Conflicting reports of chromosome numbers and differences in DNA sequences reported for *Porphyra purpurea* (Roth) C. Agardh have raised suspicion that more than one taxon has been confused under this name in the Northwest Atlantic. We have identified one of these cryptic taxa and have recently described it as a new species, *Porphyra birdiae*. Like *P. purpurea* (Roth) C. Agardh, it has an ovate to broadly elongate, foliose blade with reproductive areas segregated by a distinct line into male and female sectors. While reproductive specimens have historically been confused with *P. purpurea*, non-reproductive specimens of *P. birdiae* have been incorrectly identified as *P. umbilicalis* Kützing. Although *P. birdiae* is morphologically similar to both of these species, sequences of SSU (nuclear small subunit rRNA gene) and *rbcL* (plastid ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit gene) indicate that it is not closely related to either one. Based on *rbcL* sequences, *P. birdiae* is closely related to *P. aestivalis* Lindstrom et Fredericq, a proposed new species from Alaska.

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#### CLIMATE VARIABILITY AND PHYTOPLANKTON PRODUCTION IN PNW COAST ESTUARIES

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The west coast of North America receives strong forcing from climate modes such as El Niño-Southern Oscillation and the Pacific Decadal Oscillation. Estuaries are poised at a sensitive interface because estuarine biota and habitat will be affected by variability in properties and processes associated with the ocean, the watershed, and the local weather. Observations from the Washington coast and Willapa Bay illustrate these three arenas of influence. Variation in ocean upwelling and ocean thermocline depth associated with the 1997–98 El Niño versus the 1999 La Niña affected temperature and nutrient availability in Willapa Bay. Variation in river flow associated with the 2000–01 drought affected estuarine circulation and residence time. And, variation in prevailing wind direction and/or cloudiness was highly correlated with phytoplankton biomass. This

situation is responsible for the complexity of understanding climate impacts on estuarine systems. In order to help evaluate which mechanisms, remote oceanic processes or local watershed/estuarine characteristics, most affected Willapa Bay's phytoplankton production, several phytoplankton species were used as indicators of water mass origin and compared with primary productivity data to assess whether phytoplankton blooms were dominated by endemic or imported species. Our analysis resolved that the highest primary production (and the appearance of *Pseudo-nitzschia* spp.) was associated with oceanic intrusions of phytoplankton biomass into Willapa Bay. This result underscores the dominant role that variation in ocean and climate play in controlling Pacific Northwest estuarine production. However, while the largest blooms were oceanic in origin, numerous medium-sized production events were from either oceanic or estuarine sources, indicating a complex picture.

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#### CAPTURING THE LIGHT FANTASTIC: OCEANOGRAPHIC AND TIDAL INFLUENCES ON INTERTIDAL ALGAE

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Seaweeds experience many challenges to their persistence in intertidal zone habitats. Their growth rates must exceed losses associated with a range of ecological and physiological factors including desiccation, herbivory and wave forces. Growth rates depend on an alga's ability to capture and process light to build carbon-based molecules. We examined local (tidal height) and large (oceanographic) scale influences on algal photosynthetic efficiency and light climate, respectively. At the local scale, we combined periodic measurements of physiological state using PAM fluorometry with traditional demographic monitoring of a *Postelsia palmaeformis* population over a tidal height gradient. Parameter estimates derived from rapid fluorescence-irradiance curves were correlated with longer-term ecological performance measures including growth rate, morphology, survivorship and reproductive output. At larger scales, we made continuous in situ measurements of chlorophyll fluorescence and light attenuation in the intertidal zone at six sites during 2001 and 2002. Light attenuation to the benthos was sharply reduced at sites when chl-a fluorescence was high. Long-term, large-scale monitoring of intertidal zone chl-a and macroalgal abundances documents that striking differences among sites are persistent and associated

with oceanographic factors. The light saturation parameter and maximum photosynthetic rate calculated for several common intertidal macrophytes, along with published values of the irradiance needed to saturate their growth rates, suggest that underwater light levels may limit intertidal algal growth where phytoplankton blooms are common and persistent. We conclude that physiological stress associated with tidal and oceanographic factors contribute to macroalgal distributions.

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### CRYPTOENDOLITHIC PHOTOSYNTHETIC COMMUNITIES WITHIN RECENT AND ANCIENT TRAVERTINE IN YELLOWSTONE NATIONAL PARK.

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Travertine terraces have been deposited by calcareous hot springs in Yellowstone from as early as 365,000 years to the present. Most of these porous and non-porous CaCO<sub>3</sub> rocks (old or new) contain a 1–2 mm thick greenish band about 1–3 mm below the upper surface. These bands are composed of cyanobacteria and, sometimes, unicellular green algae. Although some moisture may be retained for much of the year, all undergo freezing in winter and desiccation in summer. DGGE (denaturing gradient gel electrophoresis), with subsequent 16S rDNA sequence analyses of bands, has shown that relatively few phylotypes of cyanobacteria are present, but some occur in travertine of very different ages, indicating secondary establishment of the communities. Clonal cultures of predominant types have also been established and sequenced. All those tested are able to survive extreme desiccation. Preliminary sequence analyses of cultures show that some strains are nearly identical to known cyanobacterial strains while others show little similarity. One sequence is 100% identical to the cyanobacterium *Cyanobium gracile*. This cyanobacterium is known to be distributed worldwide in lakes and brackish seas, but not in a cryptoendolithic environment. Another sequence shows 99% identity to two cyanobacteria isolated from Antarctic freshwater ponds. Both Antarctic ponds and Yellowstone travertine are environments in which adaptations for desiccation and/or freezing tolerance could be crucial. The lack of ecological similarity among some of these strains indicates that genes other than 16S rDNA must be used for differentiation. These results will be discussed along with the ecology of travertine habitats.

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### “PLANTAE” EMENDED BASED ON THE COMMON HISTORY OF THE PRIMARY PLASTID ENDOSYMBIOSIS

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Phylogenetic relationships between three lineages of the primary photosynthetic eukaryotes (red algae, green plants and glaucophytes) seem to remain unresolved because previous nuclear multigene phylogenies used the highly reduced red algal endosymbiont (nucleomorph) and/or incomplete red algal gene sequences. Here we carried out nuclear multigene phylogeny of various lineages of only mitochondria-containing eukaryotes, using complete sequences from the red alga *Cyanidioschyzon merolae*. Cellular slime molds were designated as the outgroup based on concatenated two paralogous tubulin gene sequences. Our phylogenetic analyses based on a 1525-amino-acid sequence of four concatenated nuclear genes (actin, elongation factor-1a, a-tubulin and b-tubulin) resolved the presence of two large robust monophyletic groups (Groups A and B) and the basal eukaryotic lineages (Amoebozoa). Group A corresponded to the Opisthokonta (Metazoa and Fungi), whereas Group B included various primary and secondary plastid-containing lineages (red algae, green plants, glaucophytes, euglenoids, heterokonts, and apicomplexans), Ciliophora, Kinetoplastida, and Heterolobosea. The red algae represented the most basal lineage within Group B. Therefore, the primary plastid endosymbiosis likely occurred once in the common ancestor of Group B, and the primary plastids were subsequently lost in the ancestor(s) of organisms which now lack primary plastids within group B. A new concept of *ÖPlantaeÖ* is proposed for phototrophic and non-phototrophic organisms belonging to Group B, on the basis of the common history of the primary plastid endosymbiosis. This new scenario of the plastid evolution is discussed in comparison with genome information recently accumulated and plastid genome comparison.

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**NEW RECORDS OF CYANOBACTERIA FROM EPILITHIC HABITATS IN THE GREAT SMOKY MOUNTAINS NATIONAL PARK**

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Great Smoky Mountains National Park is home to a diverse collection of plants and animals. The protection that the park provides, along with variations in climate, elevation, and geology supports this high biodiversity. In an effort to record the biodiversity within the park an All Taxa Biodiversity Inventory project was established in 1997. This inventory will serve as a baseline for the biodiversity in the park, and will be used to monitor future environmental impacts. Epilithic algae were collected from sites from Hen Wallow Falls and along Cataloochie Road in the Great Smoky Mountains National Park 5–6 May, 2002. Of the 54 cyanobacteria species identified from Hen Wallow Falls and Cataloochie Road sites, 41 were new species records to the park: 10 Chroococcales, 11 Oscillatoriales, and 20 Nostocales. Some of these did not fit published descriptions based on morphology and may be new to science. Of special interest are several taxa in the Nostocaceae: *Anabaena*, *Trichormus*, *Nodularia*, and *Nostoc*. A *Capsosira* species was highly unusual and undoubtedly represents a species new to science. Morphological characterization is complete, and efforts are now being made to characterize 16S rRNA and 16S-23S ITS regions of selected strains.

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**PARASITES AND PHYTOPLANKTON, WITH A SPECIAL EMPHASIS ON DINOFLAGELLATE INFECTIONS**

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Eukaryotic parasites are believed to play important roles in phytoplankton ecology and particularly in bloom dynamics of red-tide dinoflagellates. Apart from the prokaryotic parasites such as viruses and bacteria, certain flagellates and fungi have received some degree of attention as eukaryotic parasites of phytoplankton. Our understanding of fungal parasites is largely based on studies for freshwater diatoms and dinoflagellates, although fungal infections are known for some marine phytoplankton, including diatoms. By comparison, the dinoflagellate genus *Amoebophrya* and the newly described Perkinsozoa *Pavilucifera infectans* are widely distributed in coastal

waters of the world and are well known as eukaryotic parasites of dinoflagellates. Recent work indicates that these parasites have significant impacts on the ecophysiology and behavior of dinoflagellate hosts. Thus, the ecological roles of *Amoebophrya* spp. and *Pavilucifera infectans* should be carefully considered in developing concepts about plankton dynamics and material flows in marine food webs.

## 133

**STUDIES ON THE LIFE HISTORY OF THE PORTUGUESE RED ALGA *PORPHYRA DIOICA* (BRODIE AND IRVINE) UNDER VARYING ENVIRONMENTAL CONDITIONS**

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The life history of *Porphyra dioica* collected in Porto, Portugal, was investigated under laboratory conditions. This is one of the most common *Porphyra* species on the North Coast of Portugal and can be found throughout the year. Field studies showed higher percentage cover, from 23 to 66%, during February through May. Varying temperature, light intensities and photoperiods were tested. The zygospores germinated faster at 15°C, and at 25  $\mu\text{mol m}^{-2} \cdot \text{s}^{-1}$ . Growth rate of the conchocelis was affected by temperature rather than by photoperiod. In the three photoperiods tested, growth rate was always higher at 15°C, under 25 to 75  $\mu\text{mol m}^{-2} \cdot \text{s}^{-1}$ , although not significantly different from that at 20°C. Difference between these two temperatures and 5 and 10°C was significant. Conchosporangia formation was higher in 15°C and at short-day, 8:16, Light:Dark and 25 to 75  $\mu\text{mol m}^{-2} \cdot \text{s}^{-1}$  and was almost non-existent in free floating conditions. Optimal conditions for conchosporangia maturation, 15°C, 8:16, Light:Dark and 5 to 25  $\mu\text{mol m}^{-2} \cdot \text{s}^{-1}$  also promoted spore release after 18 weeks. Aeration appeared to be crucial for normal blade development. No archeospores were observed. The first findings of the optimal conditions for growth of the gametophyte stage will also be discussed.

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**CRYOPRESERVATION OF *CHLAMYDOMONAS REINHARDTII* (CHLOROPHYCEAE): A CAUSE OF LOW VIABILITY AT HIGH CELL DENSITY**

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Cryopreservation provides a convenient method for long term storage of living organisms. Current protocols allow the successful cryopreservation of a wide range of algae, although many strains remain recalcitrant to cryopreservation. *Chlamydomonas reinhardtii*, a species utilized in many molecular and biochemical studies, survives cryopreservation best at low cell density. We show that reduced viability at higher cell densities is caused by the accumulation of a substance released from *C. reinhardtii* into the culture medium during cryopreservation. A mutant strain of *C. reinhardtii* (cw10) with a greatly reduced cell wall did not release a substance inhibitory to wild type or cw10 *C. reinhardtii* during cryopreservation, and could be cryopreserved with the same viability regardless of cell density. The inhibitory substance is small (mw < 1300), polar, heat-stable and organic. *Chlamydomonas moewusii* Gerloff and *Chlamydomonas zebra* Korschikov ex Pascher both produce substances that reduce the viability of cryopreserved *C. reinhardtii*. However, neither is affected by the inhibitory substance produced by themselves or *C. reinhardtii*. *Pandorina morum* (Müller) Bory and *Volvox carteri f. nagariensis* Iyengar are colonial Volvocalean algae related to *C. reinhardtii* that cannot be successfully cryopreserved. They both generate substances that inhibit *C. reinhardtii* during cryopreservation. The identification of the substance inhibitory to *C. reinhardtii* during cryopreservation should explain why this alga cryopreserves best at a low cell density, and may lead to protocols that facilitate the more successful cryopreservation of *C. reinhardtii* and related algae.

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#### LIFE HISTORY AND ECOLOGY OF TRETEPOHLIACEAE (CHLOROPHYTA) IN THE WEST OF IRELAND

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Species of subaerial green algae of the family Trentepohliaceae are common in tropical and temperate regions. Despite nearly two centuries of investigations, several important aspects of their biology (such as life history and taxonomic relationships of some species) are still poorly understood. In western Ireland, the abundance of Trentepohliaceae is a peculiar feature of the subaerial algal vegetation. Six species are present (*Phycopeltis arundinacea*, *Printzina lagenifera*, *Trentepohlia abietina*, *T. aurea*, *T. iolithus* and *T. umbrina*). Life history and phenology of these were examined by extensive field and culture studies. In contrast to most other subaerial algae, the Trentepohliaceae show a generally strict substratum-specificity in western Ireland. *T. iolithus*, in particular, is remarkable for its occurrence on concrete walls, where it may produce extensive dark-red growths.

Our observations suggest that general statements about the life history of *Trentepohlia* should be reconsidered critically. There is no evidence that in Irish populations a regular alternation of isomorphic gametophytes and sporophytes takes place; biflagellate swimmers (usually considered gametes) behave as asexual spores and reproduce the same morphological phase. No fusion of gametes was observed and a detailed examination of the literature concerning the genus shows that this phenomenon is extremely rare. A combination of studies based on different types of data (molecular data, examination of very large numbers of field samples, chromosome numbers, culture studies) is considered fundamental to any definitive clarification of the taxonomy and life history of *Trentepohlia*.

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#### METABOLIC FLUX ANALYSIS OF HALOGENATED MONOTERPENE BIOSYNTHESIS IN MICROPLANTLET SUSPENSION CULTURES OF THE MARINE RED MACROALGAE *OCHTODES SECUNDIRAMEA* AND *PORTIERIA HORNEMANNII*

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The bioprocess engineering of marine macroalgae or "seaweeds" for the production of bioactive secondary metabolites is an emerging area of marine biotechnology. One novel metabolic theme is the biosynthesis of halogenated monoterpenes by red macroalgae. Specifically, two species of tropical marine red macroalgae, *Ochtodes secundiramea* and *Portieria hornemannii*, contain halogenated monoterpenes that serve as a chemical defense to herbivory. Some of these halogenated monoterpenes also possess potent activity against several human tumor cell lines. Field collections of *O. secundiramea* and *P. hornemannii* exhibit site-to-site variations in halogenated monoterpene (HMT) content. In contrast, microplantlets of *O. secundiramea* and *P. hornemannii* established through cell and tissue culture techniques have remarkably similar HMT profiles when cultivated in a photobioreactor under identical, controlled conditions. Both cultures share Apakaodotene B as the only cyclic HMT, 10E-bromomyrcene and 10E-bromo-3-chloro-a-myrcene as the dominant acyclic HMTs, and myrcene as their common precursor. Furthermore, halogenated monoterpene biosynthesis in red macroalgae has three common manifolds: elaboration of myrcene from geranyl diphosphate by a novel marine monoterpene synthase, regio- and stereoselective bromination of myrcene by a novel marine halogenase, and bromonium-ion assisted cyclization and halogenation of myrcene. Two quan-

titative tools of metabolic engineering – metabolic flux analysis and metabolic pathway analysis – were used to elucidate the factors that control halogenated monoterpene biosynthesis. By coordinated perfusion of nutrients, bromide, and key enzyme co-factors to the photobioreactor culture, both the yield and distribution of halogenated products can be externally directed to target compounds. These efforts represent the first application of metabolic engineering principles to the manipulation of secondary metabolism in macrophytic marine organisms.

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### GAMETE RECOGNITION AND SEXUAL ISOLATION IN A RED ALGA, *AGLAOTHAMNION BYSSOIDES* (RHODOPHYTA)

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The binding of fluorescein isothiocyanate (FITC) conjugated lectins to gametes of *Aglaothamnion byssoides* Itono during the fertilization was studied by the use of confocal microscope. The physiological effects of lectins and carbohydrates on gamete binding were also examined. Three lectins, concanavalin A (ConA), Soybean agglutinin (SBA) and wheat germ agglutinin (WGA) bound to the surface of spermatia, but each lectin labeled different region of the spermatium. SBA bound only to the spermatial appendages but ConA bound to the whole spermatial surface except spermatial appendages. WGA labeled narrow region which connects spermatial body and appendages. During fertilization, ConA and WGA specific substances on the spermatial surface moved towards the area contacting with trichogyne and accumulated on the surface of fertilization canal. Spermatial binding to trichogynes was inhibited by pre-incubation of spermatia with SBA, while trichogyne receptors were blocked by the complementary carbohydrate, N-acetyl-D-galactosamine. WGA and its complementary carbohydrate had little effect on gamete binding. For searching the step of sexual isolation, crossing experiment was performed between *Aglaothamnion byssoides* and twelve other red algal species. Results showed that the gamete recognition was genus-specific: the gametes bound freely with their partners of the same genus. When two species from same genus were crossed, sexual isolation occurs gradually during the fertilization process. Therefore, sexual isolation in red algae appears to be determined by multi-step process and gamete binding is the initial step.

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### MOLECULAR EVOLUTION OF GLUTAMINE SYNTHETASE IN PROTISTS

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Assimilation of nitrogen into structural and functional macromolecules is a critical process in all living organisms. Glutamine synthetase (GS) catalyzes the ATP-dependent condensation of NH<sub>4</sub><sup>+</sup> and glutamate thus coupling nitrogen assimilation with carbon metabolism. The GS superfamily is comprised of three gene families: GSI, GSII, and GSIII. Representatives of the three gene families are found in prokaryotes, suggesting the genes are paralogous. In contrast, knowledge of the distribution and evolution of the GS gene families in eukaryotes is limited. The endosymbiotic origin of plastids is well established. Phylogenetic analyses of genes distributed among photosynthetic and non-photosynthetic organisms may provide insight into the timing and stability of the endosymbiotic events. GSII has been characterized from a variety of prokaryotic and eukaryotic species, including the stramenopiles, *Skeletonema costatum* and *Phytophthora infestans* (AAN31463). I evaluated several a priori hypotheses regarding the placement of the stramenopile GSII sequences in addition to performing unconstrained analyses. In all analyses, GSII from *S. costatum* and *P. infestans* grouped together. Also, there was strong to moderate support for a 'protist + plant' clade, which included the vascular plants, photosynthetic algae, and the non-photosynthetic *P. infestans*. Although it remains unresolved whether GSII was transferred from the nucleus of the endosymbiont early in the evolution of stramenopiles, the continued accumulation of GSII sequences from diverse eukaryotes should allow us to rigorously evaluate this hypothesis in the future.

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### TAXONOMIC RE-EXAMINATION OF EIGHT SPECIES OF *NITELLA* (CHARALES, CHAROPHYCEAE) FROM ASIA, AND PHYLOGENETIC RELATIONSHIPS WITHIN THE GENUS BASED ON *RBCL* AND *ATPB* GENE SEQUENCES

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Eight taxa of *Nitella*, collected from Asia, were investigated using LM and SEM of the oospores and



sequencing of *rbcL* gene to improve understanding of their taxonomic status. Our SEM observations demonstrated that the oospore morphology of six taxa – *N. capitulifera* (= *N. acuminata* f. *capitulifera*), *N. japonica* (= *N. furcata* f. *japonica*), *N. tumulosa* (= *N. furcata* f. *tumulosa*), *N. megaspora* comb. nov. (= *N. pseudoflabellata* f. *megaspora*), *N. gracillima* (= *N. gracilis* f. *gracillima*), and *N. axilliformis* (= *N. translucens* f. *axilliformis*) – is distinctly different from that of the species to which R.D. Wood assigned them as infraspecific taxa. Our *rbcL* sequence data showed that *N. japonica* is separated phylogenetically from *N. furcata*, *N. tumulosa* from *N. furcata*, *N. megaspora* from *N. pseudoflabellata*, and *N. axilliformis* from *N. translucens*. In addition, to re-examine the taxonomic system of *Nitella* proposed by R.D. Wood, who treated oospore wall ornamentations as a diagnosis at the infraspecific level, we carried out molecular phylogenetic analyses using the combined sequence dataset for *atpB* and *rbcL* genes of these eight species, as well as twelve species of *Nitella* previously studied. The combined sequence data resolved five robust clades within the subgenus *Tieffallenia* that were characterized by differences in oospore wall ornamentation. However, the species and sectional diagnoses of R.D. Wood, such as the form and cell number of dactyls in vegetative thalli, were variable within the clades. Therefore, R.D. Wood's taxonomic system appears unnatural, at least within the subgenus *Tieffallenia*.

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**ECOPHYSIOLOGY OF THE RED ALGA PORPHYRA UMBILICALIS KÜTZING (RHODOPHYTA, BANGALIES): RESPONSES TO ABIOTIC STRESS**

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The effects of tidal elevation, emersion, sun exposure, and season on several antioxidant enzymes (ascorbate peroxidase, glutathione reductase, and catalase), pigments (phycoerythrin, phycocyanin, chlorophyll a and total carotene) and photosynthetic efficiency of photosystem II (Fv/Fm) in *Porphyra umbilicalis* were evaluated. Plants were collected monthly from sun-exposed and shaded locations in the high, mid, and low intertidal following periods of tidal emersion ranging from 0–6 hours. Glutathione reductase activity was greatly affected by emersion during summer months, while ascorbate peroxidase and catalase activities showed no seasonal patterns. Differences in glutathione reductase and catalase levels occurred between sun-exposed and shaded plants in the high and mid intertidal during summer. At all elevations, photosynthetic pigments showed a

strong seasonal trend, with the effect of sun exposure being most apparent during summer. While total carotene increased with emersion during summer months, the combined effects of emersion and season were inconsistent for phycoerythrin, phycocyanin and chl a. Photosynthetic efficiency (Fv/Fm) decreased following emersion in summer and fall. During most months, sun exposed plants had lower Fv/Fm values compared to plants growing in the shade. This study emphasizes the importance of examining the effects of abiotic stresses simultaneously in order to reveal interactive relationships.

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**NEW ENTITIES IN THE ORDER CHAETOPELTIDALES**

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Two species of microfilamentous green algae are added to the Chaetopeltidales, one of which is new to science. The new taxon, to be placed in a new genus and species, is described from a freshwater environment in Argentina, and is placed in the Chaetopeltidales on the basis of its ultrastructural features. Thalli are prostrate, composed of radiating branched filaments that coalesce to form a disc. The pyrenoid is circled by starch plates and traversed by one or two convoluted cytoplasmic channels. The cell walls lack plasmodesmata. Asexual reproduction takes place by means of quadriflagellate zoospores released from central cells of the thallus. The absolute configuration of the zoospore flagellar apparatus is essentially identical to those of other chaetopeltidalean algae. *Leptosiroopsis torulosa* Jao, 1937, isolated from New Zealand freshwaters, is placed in the Chaetopeltidales on the basis of its position in DNA sequence analyses. Thalli consist of erect branched filaments, the cells of which are connected by cross walls without plasmodesmata. The single large pyrenoid surrounds the nucleus. These are the first truly filamentous green algae to be included within the Chaetopeltidales.

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**INSIGHT INTO THE MITOCHONDRIAL GENOME OF EMILIANIA HUXLEYI (HAPTOPHYTA)**

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*Emiliania huxleyi* (Lohmann) Hay et Mohler 1967 (CCMP373) is the most abundant representative of the Haptophyta, and can be found in oceanic and neritic waters from subpolar to tropical latitudes. Blooms of this coccolithophorid may reach cell densities of  $2.106 \text{ ml}^{-1}$  and emit vast amounts of DMS (dimethyl sulfide), with the potential to affect the global climate. We report here the DNA sequence of more than 21 kb of the mitochondrial genome of this species, out of an approximate total of 30 kb. Preliminary annotation of the genome using database searches identified at least 16 genes. The data were also compared to the unpublished mitochondrial genome of *Pavlova lutheri*, another member of the haptophytes, and some important differences were identified. Although the gene content of *E. huxleyi* mtDNA seems to closely resemble that of *P. lutheri* mtDNA, the gene order differs substantially. Access to the *P. lutheri* and *E. huxleyi* mitochondrial genomes will permit the comparative analysis of two deeply diverging members of an ancient and ecologically significant lineage. Among other potential applications, the data will help to clarify phylogeny within the haptophytes as well as to determine the phylogenetic position of this division in relation to other groups of algae, such as heterokonts, dinoflagellates and cryptophytes.

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#### COALESCENCE DURING EARLY RECRUITMENT OF MAZZAELLA LAMINARIOIDES

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Coalescence in seaweeds is known to occur in the laboratory among young and older sporelings and in the field between neighboring conspecific clumps. However, because spores and germlings are difficult to study in the field, it is as yet unknown at which stage of population development coalescence is most important. Since many seaweeds disperse aggregated propagules, often with a sticky mucilaginous envelope around the spores, aggregated recruitment and coalescence might be more important at early stages of population establishment than among fully grown, well established clumps. Using recruitment plates maintained during several experimental times in the field, we are evaluating the above idea with mid-intertidal populations of *Mazzaella laminarioides*. During high fertility seasons, close to 45% of the spores settling within or at close (<1 m) distances of the bed exhibited aggregated recruitment, forming groups of 2 to 150+ spores. The probability of aggregated recruitments is a function of dispersal distance and spore density. The number of sporelings produced is a function of spore density and coalescence. Highest

after-recruitment mortality (first 15 days) occurs among solitary recruits, followed by sporelings conformed by small number of spores (2–4). Approximately 50% of the spores recruited, isolated or in group, coalesce within these 15 days, gradually forming massive sporelings with increasingly larger basal areas. Thus, after recruitment, sporelings may disappear (die), survive or coalesce. These three alternatives are integrated in a new demographic model for coalescing seaweeds (supported by grant FONDECYT 1020855).

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#### USE OF FOURIER TRANSFORM INFRARED (FT IR) DIFFUSE REFLECTANCE SPECTROSCOPY AND HAMMING DISTANCES TO STUDY THE PHYCOCOLLOID CHEMOSYSTEMATICS OF THE RED ALGAE (RHODOPHYTA)

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The use of red algal polysaccharide cell wall structures as taxonomic indicators has been a recurring theme in the literature for over 58 years. Traditionally the polymeric phycocolloids of the red algal cell walls have been classified into definite structures (carrageenans and agars) based on the phycocolloids present. However, this method has some obvious shortcomings, such as its inability to assign hybrid structures (carragars), or the appearance of agarocolloids in traditional 'carrageenophytes'. Thus, there is a need to develop an unbiased method of examining phycocolloid structures without assigning them names. Fourier transform infrared diffuse reflectance spectroscopy was used to analyze the spectra of a large number of red algal species, belonging to several orders. The spectra for each species resulted in a series of 'peaks' corresponding to certain wavenumbers ( $\text{cm}^{-1}$ ), which in term correspond to phycocolloid bonds. On the basis of shared/similar peaks, irrespective of their combinations to describe polymeric units, a series of matrices were developed for each family. These were coded as a 0 and 1, indicating the presence or absence of a peak, respectively. Using the concept of vector addition and Hamming distances, a methodology was formulated for analyzing ordinal and familial level groupings. Initial analysis on 173 species, representing 39 families and 12 orders of the Rhodophyta, proved to be promising. Some results corroborated previous studies, while others proved to be informative and raised the need for further chemosystematic studies. This provides the impetus for continuing to develop this method as a contribution to red algal systematics.

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### PRELIMINARY RESULTS OF CYTOSKELETAL COMPONENTS IN VARIOUS RED ALGAE USING CONFOCAL LASER SCANNING MICROSCOPY

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Data concerning the cytoskeletal components of red algae are scant. The goal of this ongoing comparative survey is to develop a more complete characterization of the red algal cytoskeleton, and subsequent elucidation of its function, using representative taxa belonging to major evolutionary lineages within the Rhodophyta. Preliminary data were obtained using enzyme digestion of cell walls and detergent rinses with direct (phalloidin) and indirect (monoclonal antibodies) labeling methods for microfilaments and tubulin, respectively. Samples were viewed using confocal laser scanning microscopy. Results will be discussed in light of the more thoroughly understood cytoskeletal system reported for green algae.

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### METHODOLOGICAL PROBLEMS OF MARINE MACROALGAE AGE ESTIMATION

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To know the birthdate of any organism is the best way to estimate its age. Unfortunately direct chronometric method, being the most reliable, can't be generally applied. In these cases indirect methods are used. One of them – morphobiometric – is used to estimate age of trees by the number of year rings in the stem or furoid algae by the number of dichotomic branching. Both methods are widely used however the data obtained may deviate considerably from the true age of the plants. In this study I compared chronometric data on algal age to those obtained by calculation of the number of dichotomic branching in *Fucus evenescens* growing in Kamchatka, Russia. Field experiments were carried out from 1999 to 2002. No marked with plastic labels plants survived for 3 years. The comparison showed that morphobiometric data exceeded true age of algae at least in 2–3, sometimes even in 5 times. The first year plants can produce up to 5 dichotomous branching per season and can become fertile in the first months of their life. There is information in Russian literature that Kamchatka's *F. evenescens* age can reach 12–15 years. I consider these data erroneous and suppose underestimation of the number of branches in the early stages of *Fucus* development

to be the cause of overestimation of its true age. In my opinion it is not more than 4–5 years. Application of morphobiometric method is more problematic in case of polytomy. Besides that this method usually causes damage or death of the plant. So I consider that only chronometric method can give the researcher exact information on the age of macroalgae.

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### CONTROLLING HARMFUL ALGAL BLOOMS THROUGH CLAY FLOCCULATION

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The potential use of clays to control harmful algal blooms (HABs) has been explored in East Asia, Australia, the United States, and Sweden. In Japan and South Korea, minerals such as montmorillonite, kaolinite, and yellow loess, have already been used in the field effectively, to protect fish mariculture from *Cochlodinium* spp. and other blooms. Cell removal occurs through the flocculation of algal and mineral particles, leading to the formation of larger aggregates (i.e. marine snow), which rapidly settle and further entrain cells during their descent. In the U.S., several clays and clay-rich sediments have shown high removal abilities (e.g. >80% cell removal efficiency) against *Karenia brevis*, *Heterosigma akashiwo*, *Pfiesteria piscicida* and *Aureococcus anophagefferens*. In some cases, the removal ability of certain clays was further enhanced with chemical flocculants, such as poly-aluminum chloride (PAC), to increase their adhesiveness. However, cell removal was also affected by bloom concentration, salinity, and mixing. Cell mortality was observed after clay addition, and increased with increasing clay concentration, and prolonged exposure to clays in the settled layer. Mesocosm, field enclosure, and flume experiments were also conducted to address cell removal with increasing scale and flow, water-column impacts, and the possible benthic effects from clay addition. Results from these studies will be presented, especially those in regards to water quality, seawater chemistry, bottom erodibility and faunal impacts in the benthos. At this time, clay dispersal continues to be a promising method for controlling HABs and mitigating their impacts based on existing information and experimental data.

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### LIFE HISTORY AND FEEDING PREFERENCES OF GALLERY-FORMING HARPACTICIDS IN DICTYOTALEAN SEAWEEDS

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The harpacticoid copepod *Dactylopusioides macrolabris* has been reported to make galleries beneath the cuticle of cortical cells of *Dictyota dichotoma* (Dictyotales, Phaeophyceae; Green 1958). Besides this species, we have found several more harpacticoids from Japanese coasts that have similar habits, infesting various dictyotalean species. In laboratory culture experiments growing the harpacticoids for several generations and recording their behaviors by interval video recoding, we have found that these harpacticoids spend most of the lives in the galleries, feeding only on the algal tissues. Different species showed different feeding preferences even among morphologically very close species: one species fed most of the dictyotalean species examined, whereas another species could digest only one species. In order to discuss the evolutionary interactions between the harpacticoids and host seaweeds, comparative molecular phylogenetic analyses of the harpacticoids and host seaweeds were studied using rDNA ITS2 (harpacticoids) and *rbcL* (Dictyotales) DNA sequences.

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#### **DISTRIBUTION AND INVASION OF *BANGIA ATROPURPUREA* (RHODOPHYTA) IN THE LAURENTIAN GREAT LAKES**

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Müller et al. (1998) noted that freshwater collections of the genus *Bangia* formed a distinct group separate from marine entities in gene sequence analyses. Recently, the species epithet *B. atropurpurea* has been resurrected to represent this freshwater lineage. This taxon is one of many invasive species within the Laurentian Great Lakes. *B. atropurpurea* was first observed in Lake Erie in 1964 and by 1982 was observed in all of the Great lakes except Lake Superior. The present study was initiated to examine the further spread of *B. atropurpurea* and determine the origin of these populations. Hence, a survey of all the Great Lakes was conducted in 1995 (86 sites) and again in 2002 (104 sites). *Bangia* was observed at 43 sites in 1995 and 39 sites in 2002. For the first time, this alga has been observed to be present in the St. Lawrence River (1995), Georgian Bay on Lake Huron (2002) and Lake Simcoe (eastern shore, 2002) and hence this alga appears to be spreading into new locations. Cluster analyses of morphological data reveal three distinct groupings that do not separate according to location or lake basin. Preliminary analyses of ITS 1 and 2 sequences show

differences among samples within Lake Ontario and among all Lakes; however, collections from Lake Simcoe are very similar in sequence. We are continuing to examine the relationship of Great Lakes populations with freshwater collections from Europe.

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#### **PHYLOGENETIC SYSTEMATICS AND PHENOLOGY OF THE HAWAIIAN ENDEMIC FRESHWATER RED ALGA, *BATRACHOSPERMUM SPERMATIOPHORUM***

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The only known population of *Batrachospermum spermatiophorum* Vis et Sheath, located in a small stream on east Maui, Hawaii, was sampled from November 2001 to October 2002 to investigate its phenology and taxonomy. Phylogenetic analyses of the *rbcL* gene for *B. spermatiophorum* confirm its placement within the Section Contorta. Comparison with *rbcL* gene sequences for all other available *Batrachospermum* species illustrates that *B. spermatiophorum* is distinct in its *rbcL* nucleotide sequence and should be maintained as a separate species. The phenology of *B. spermatiophorum* differed from similar studies on temperate *Batrachospermum* species in that gametophytes were present year round, and no "chantransia" stages were identified at any point during the study. Maximum plant length was highest in late fall and early winter months, while gametangial production peaked in February and March. Carposporophyte abundance rose sharply in July and August, and the percent of carposporophytes producing carpospores was highest in late summer and early fall months. The year-round presence of gametophytes may be a result of fewer seasonal cues in the tropical environment than occur in temperate locations.

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#### **PHYLOGENETIC ANALYSIS OF THE SUBGENUS *EUGLENA* WITH PARTICULAR REFERENCE TO THE TYPE SPECIES *EUGLENA VIRIDIS* (EUGLENOPHYCEAE)**

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*Euglena viridis* was first described by Antony van Leeuwenhoek in 1674. This taxon later became the

type for the genus *Euglena* erected by Ehrenberg in 1838. The primary characters that distinguish this taxon are the single stellate chloroplast and spherical mucocysts. A number of related *Euglena* species are similar in size, bear one or two stellate plastids and possess spherical or spindle-shaped mucocysts. We conducted morphological and molecular studies on taxa in the subgenus *Euglena* (all of which bear stellate chloroplasts) and compared this to genera in the subgenus *Calliglena* (non-stellate chloroplasts). Morphologically the strains in subgenus *Euglena* were very similar, except for chloroplast number and mucocyst shape. The *E. stellata* group has one chloroplast and a distinctive spindle-shaped mucocyst; the *E. geniculata* group has two chloroplasts and spherical mucocysts; the *E. viridis* group has one chloroplast and spherical mucocysts. Molecular analyses using SSU and LSU rDNA demonstrated that the subgenus *Euglena* is not monophyletic. The combined SSU/LSU trees provide strong support for a stellate clade (subgenus *Euglena*), but one strain of *E. viridis* diverges at the base of the *Euglena*/*Calliglena* lineage. Multiple subclades are found within the main stellate clade. *E. tristellata* forms a separate divergence and four *E. stellata* strains form a single, well-supported subclade. Two *E. viridis* strains are among the *E. geniculata* group clade, while six others form two separate, but well-supported clades. This study demonstrates that the type species, *E. viridis*, is paraphyletic and will need to be redefined.

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#### NON-INDIGENOUS AND INVASIVE MARINE ALGAE IN HAWAII

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Histories, patterns of introduction, management of impacts and predictable futures? Ecological impacts of weedy introduced and native marine algae have set Hawai'i apart from elsewhere in the tropics and offers a new show case for macroalgal blooms. Partly because of our isolation, impacts of non-indigenous species have been clearly identified, in some cases almost as quickly as introductions have occurred to Hawai'i. While hull fouling or intentional introductions may have been initial mechanisms for macroalgal introductions, spread among the Hawaiian Islands is not necessarily linked to the vector for arrival. Further, impacts of non-indigenous species are also not fully predictable, prior to introduction. This situation leads to challenging gaps in management strategies because few generalizations about the roles of nutrients or herbivory as drivers for macroalgal blooms can be supported at this time. Weedy native species represent a wide range of alternate impacts with other apparent combinations of forcing functions. Case histories for the most significant non-

indigenous and weedy native algae will be presented with emphasis on potential new criteria to assess ecological impacts and provide resource biologists with critical new tools for the management of reef ecosystems.

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#### INVASIVE MACROALGAE ON TROPICAL REEFS: IMPACTS, INTERACTIONS, MECHANISMS AND MANAGEMENT

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The introduction of non-indigenous species is currently viewed as one of the largest threats to global biodiversity. Non-indigenous marine algae (NIMA) in temperate seas around the world have caused significant losses to ecosystem structure and function. However, the introduction of invasive species in tropical marine ecosystems has not typically been viewed as a significant threat despite the number of species that have been transported to reef regions around the world for open-reef aquaculture. The research presented here represents some of the first quantified evidence of significant negative impacts of NIMA in tropical waters. This study characterized several ecological and physiological aspects of one the most successful and potentially threatening NIMA on Hawaii's coral reefs, *K. alvarezii*. Results of large-scale surveys and a number of permanently established invader removal plots suggest that *K. alvarezii* is having negative impacts on native species diversity. Interactions between *K. alvarezii* and coral abundance were examined using photoquadrats and results indicate that the invader is causing coral death as a result of overgrowth and shading. Possible mechanisms influencing invader success including responses to nutrient enrichment, grazer consumption rates and reproductive characteristics were examined. Results from a number of experiments suggest that without mitigation *K. alvarezii* will continue to spread. In an effort to minimize negative impacts and prevent spread several management strategies were examined including manual removal, use of chemical and temperature treatments and enhancement of native sea urchins. While some of these tools are promising control options, rapid implementation is needed to prevent further damage.

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#### DEVELOPMENTS ON TWO NEWLY CULTIVATED SPECIES *CAPSOSIPHON FULVESCENS* AND *COSTARIA COSTATA* IN KOREA

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In the recent years, there has been increasing demand of nutritive and health foods in Korea. Species diversification is needed in order for seaweed aquaculture to take advantage of these expanding markets. With this in mind, the cultivation of *Capsosiphon fulvescens* and *Costaria costata*, was successfully developed. *Capsosiphon fulvescens* is a filamentous chlorophycean alga growing in the upper littoral regions of the rocky-shore. It has been used as food in the form of soup with oysters along the Southwestern Coast of Korea. After the development of artificial seed production (control of light, photoperiod and temperature of parthogenetic gametes), pilot scale, and then large scale, cultivation techniques were established for mass culture of this species. In 2001, the retail price was about \$6.2/kg fresh wt with a total production of 770 ton. *Costaria costata*, a phaeophycean alga, was initially cultivated along the Eastern Coast of Korea. It has now been transplanted successfully to culture grounds along the Southern Coast. The cultivation techniques are very similar to *Undaria* and *Laminaria*, ie. long line techniques. Its price is about \$1.7/kg fresh wt with total production of 2,000 ton. An overview of these new maricultures technologies that have lead to the successful expansion of the seaweed industry in Korea will be presented.

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#### MAPPING FLOATING KELP BEDS IN ALASKA WITH REMOTE SENSING

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The commercial harvest of floating kelps has promise as a business opportunity that could be especially valuable for small communities in southeast Alaska. The giant kelp, *Macrocystis*, is harvested mainly for the commercial herring roe-on-kelp harvest in Alaska. In addition, in southeast Alaska the two other species of floating kelps, *Nereocystis luetkeana* and *Alaria fistulosa*, have been commercially harvested since 1992 for use as agrochemicals by the Alaska KelpCo. The continued existence of this company and others that may wish to harvest seaweeds on a commercial basis is threatened by the lack of a kelp harvest management system in Alaska. The objective of this research is to develop a reliable and cost effective method for estimating the aerial extent and the standing crop biomass of the floating kelps in SE Alaska. We are employing an aerial multispectral imaging system that can be flown at varying altitudes to achieve spatial resolutions ranging from 0.5 to 2 meters. We are investigating whether the three species have unique spectral or textural characteristics in the multispectral imagery that can be used to differentiate the populations. The end products for this project will be kelp resource maps that delineate the spatial extent and

biomass of kelp populations within the beds and a methodology for assessing kelp beds with mixed species composition. These products will find use by resource managers such as the Alaska Department of Fish and Game and the Department of Natural Resources.

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#### EFFECTS OF LIGHT AND NUTRIENTS ON PERIPHYTON COMMUNITY COMPOSITION IN A HAWAIIAN STREAM

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Streams in Hawaii are experiencing increasing anthropogenic impacts due to agriculture and urbanization via nutrient enrichment and riparian canopy removal. The effects of these factors on stream algal communities in Hawaii have rarely been studied using manipulative experiments. A study was conducted in a slightly impacted forested stream on Oahu, Hawaii, to examine the effects of nutrient enrichment and canopy cover on the benthic algal community, especially diatoms. Benthic algae were colonized onto nutrient-diffusing substrata in a factorial design with nitrate and phosphate enrichments, at high and low light levels at similar elevations. Algal productivity as measured by ash-free dry mass and chlorophyll a levels increased significantly with nitrate enrichment at the high light site. Pulse-amplitude modulated (PAM) fluorescence was used to estimate photosynthetic parameters in situ, and indicated a higher maximum photosynthetic rate for the algal community with nitrate enrichment at the high light site. Significant differences in diatom community composition were observed at low and high light sites, and several diatom species also responded significantly to either nitrate or phosphate enrichment at both sites. These results suggest that benthic stream algal communities in Hawaii may be significantly affected by human activities within watersheds.

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#### EXAMINING PHYTOPLANKTON COMMUNITIES WITH ENVIRONMENTAL PCR

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Recent advances in biotechnology have taken much of the guesswork out of many molecular biological procedures, and promote a high rate of success even for relative beginners. In designing the laboratory component of my Phycology course, I have taken advantage of these improved methods to develop a semester-long, molecular-based class project, using

“environmental PCR” to examine phytoplankton diversity. Students learn a number of common molecular techniques within the context of dynamic and unrehearsed phycolgical research. This provides a greater appreciation of the general utility of molecular technology, and its application to algal systems in particular. A series of easy to follow protocols are linked together, allowing students to PCR amplify sequences from mixed environmental samples, clone the resulting fragments to separate individual sequences, screen the clones by restriction enzyme digests and sequence clones with clearly different restriction patterns. The resulting sequences then are used in BLAST searches of GenBank to determine the nearest available match among sequences annotated previously. The techniques allow both qualitative and quantitative estimates of the organisms present, as well as comparative analyses of the diversity observed through direct visual observations versus those recovered by PCR. Each step of the procedure can be accomplished in the duration of a typical 3 hour lab period, and most involve intervals of incubation that permit adequate time for other lab exercises, such as a survey algal diversity, on which traditional phycolgical laboratories are based.

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#### **ALGAL TURF COMPOSITION AT TWO COASTAL LOCATIONS ON THE ISLAND OF HAWAI'I**

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Algal turfs, or mats of interwoven multispecific algal 1–3 cm in height, play an important role in the reef community in Hawai'i, and yet they are often overlooked. Two sites on opposite coasts of the island (Richardson's Beach Park and Puako) were sampled from September 2000 to July 2002. The samples from quadrats along transect lines were analyzed to find percent abundance of each species. Specimens were examined microscopically to generate a species list. Over 50 different species of algae were identified, and red algae (Rhodophyta) dominated the samples at both sites. Several new records for the island of Hawai'i were found. Algal turf species diversity ( $H'$ ) varied between sites and among sampling dates. The most abundant species showed aggregated or patchy spatial patterns in the turf.

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#### **INCERTAE SEDIS NO MORE: THE PHYLOGENETIC AFFINITY OF HELICOSPORIDIA**

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The Helicosporidia are a unique group of pathogens found in diverse invertebrate hosts. They have been considered to be either protozoa or fungi, but have remained incertae sedis since 1931. Following the isolation of a *Helicosporidium* sp. in Florida, we showed that the Helicosporidia are non-photosynthetic green algae. Phylogeny reconstruction inferred on several housekeeping genes (including actin and b-tubulin) consistently and stably grouped *Helicosporidium* sp. among member of Chlorophyta. Additionally, nuclear SSU rDNA phylogenies identified *Helicosporidium* as a sister taxa to another parasitic, non-photosynthetic algal genus: *Prototheca* (Chlorophyta, Trebouxiophyceae). Comparison of mitochondrial (*cox3*) and chloroplast (*rrn16*) genes confirmed that *Helicosporidium* and *Prototheca* have arisen from a common photosynthetic ancestor, and suggested that Helicosporidia contain *Prototheca*-like organelles, including a vestigial chloroplast (plastid). In an effort to better characterize the biology of *Helicosporidium* sp., a cDNA library has been constructed and expressed sequences tags (ESTs) have been generated. Most of these ESTs exhibited similarity with algal and plant genes. Significantly, the EST library provided with additional evidence that *Helicosporidium* sp. does have a plastid, as numerous nuclear-encoded, plastid-targeted genes were identified. Characterization of the plastid is currently underway: several chloroplast-like genes (*rrn16*, *rrn23*, *tufA*) have been cloned and their sequences are being used to isolate the entire plastid genome. Similar to the situation described in *Prototheca*, the *Helicosporidium* plastid may be reduced, as most of the genes involved in photosynthesis likely have been lost during the course of evolution.

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#### **COPEPODOLOGY FOR THE PHYCOLOGIST WITH APOLOGIES TO G. E. HUTCHENSON**

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*Heterocapsa triquetra* is one of the most common bloom forming dinoflagellates found in estuaries and near shore regions around the world. In order to bloom, *H. triquetra* optimizes a suite of factors including low grazing pressure, increased nutrient inputs, alternative nutrient sources, and favorable salinity and hydrodynamic conditions, as well as the negative factors of temperature-limited growth, short day lengths, and periods of transient light limitation. The prevailing environmental conditions associated its wintertime blooms are largely the result of atmospheric forcing. Low-pressure systems moved through coastal area at frequent intervals and are accompanied by low air temperatures and rain-

fall. Runoff following the rainfall events supplies nutrients critical for bloom initiation and development. *Heterocapsa triquetra* blooms can reach chl a levels  $> 100 \text{ mg L}^{-1}$  and cell densities between 1 to  $6 \times 10^6 \text{ L}^{-1}$ . As the blooms develop, nutrient inputs from the river became insufficient to meet growth demand and *H. triquetra* feeds mixotrophically, reducing competition from co-occurring phytoplankton. Cloud cover associated with the low-pressure systems light limit *H. triquetra* growth as do low temperatures. More importantly though, low temperatures limit micro and macrozooplankton populations to such an extent that grazing losses are minimal.

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**THE RECENT SPREAD OF AN INVASIVE KELP, *UNDARIA PINNATIFIDA*, IN CALIFORNIA: HOW SIMILAR IS THIS INVASION TO OTHER *UNDARIA* INVASIONS WORLDWIDE?**

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Invasive algal species have the potential to change the structure and ecology of native algal communities. One well-known invader, the large Japanese kelp *Undaria pinnatifida*, has recently become established at several locations along the central and southern California coast (Monterey, Santa Barbara, Catalina, and others). Previous to its introduction in the northeastern Pacific, *Undaria* has become established along the coastlines of several countries, including Australia, New Zealand, Argentina, England, and France. However, the seasonal population dynamics, rate of spread, and impact on local communities at each invasion site varies. *Undaria* in the Santa Barbara, CA harbor exhibits two distinct recruitment pulses per year (fall, late winter), with nonoverlapping generations of adult individuals. Individuals can grow rapidly and become reproductive a month after appearing as recruits (2–3 cm long), indicating a potential for rapid spread. However, *Undaria* may be effectively controlled by grazing via natural recruitment of the kelp crab *Pugettia producta*. However, *Undaria* invasions in other California invasions have not been controlled by herbivory, and *Undaria* populations in these areas have the potential to compete with a wide diversity of native California kelp species for habitat space and light.

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**INTERACTIONS BETWEEN PLANKTONIC MICROALGAE AND PROTOZOAN GRAZERS**

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For an algal bloom to develop, the growth rate of the bloom-forming species must exceed the sum of all loss processes. Among these loss processes, grazing is generally believed to be one of the more important factors. Based on numerous field studies it is now recognised that microzooplankton are dominant consumers of phytoplankton in both open ocean and coastal waters. Heterotrophic protists, a major component of microzooplankton communities, constitute a vast complex of diverse feeding strategies and behaviour which allow them access to even the larger phytoplankton species. A number of laboratory studies have shown the capability of different protistan species to feed and grow on bloom forming algal species. Because of short generation times, their ability for fast reaction to short-term variation in food conditions enables phagotrophic protists to fulfil the function of a heterotrophic buffer, which might balance the flow of matter in case of phytoplankton blooms. The importance of grazing as control of microalgae becomes most apparent by its failure; if community grazing controls initial stages of bloom development, there simply is no bloom. However, if a certain algal species is difficult to graze, e.g. due to specific defence mechanisms, a reduced grazing pressure will certainly favour bloom development. The present contribution will provide a general overview on the interactions between planktonic microalgae and protozoan grazers with special emphasis on species-specific interactions and algal defence strategies against protozoan grazers.

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**IDENTIFICATION OF EUGLENOIDS THAT PRODUCE ICHTHYOTOXIN(S) (EUGLENOPHYTA)**

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Diatoms, dinoflagellates, pelagiophytes, prymnesiophytes, and cyanobacteria are the only divisions of microalgae known to produce toxins. We now report toxin production by freshwater members of the genus *Euglena*. Fish mortalities (sheepshead minnows, catfish, striped bass, and tilapia) have been observed following exposure in the field to *Euglena* blooms and in the laboratory when exposed to unialgal isolates of two species of *Euglena* (*E. sanguinea* Ehrenberg and *E. granulata* (Klebs) Lemm.). Three toxic fractions have been isolated from unialgal isolates of both species, and include both water soluble and lipophilic



compounds having ichthyotoxic activity. The toxins are stable at  $-80^{\circ}\text{C}$  for at least 60 days and are heat stable to  $30^{\circ}\text{C}$ . Erratic swimming behavior of fish suggests a neurological toxin. This is the first report of fish kills by any freshwater algal taxa from both field and laboratory studies.

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### CHEMICAL ANTIHERBIVORE DEFENSES OF TEMPERATE GREEN MACROALGAE

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Many temperate green macroalgae contain secondary metabolites that provide protection from grazing by some herbivores. These include the production of dopamine hydrochloride by the ulvoid green alga *Ulvaria obscura* and the production of dimethylsulfo- niopropionate (DMSP) by many species of Ulvales and Caulerpales. The dopamine hydrochloride defense was isolated using bioassay-guided fractionation and is effective against sea urchins (*Strongylocentrotus droebachiensis*) and littorinid snails (*Littorina sitkana*). The DMSP activated defense system involves enzymatic cleavage of DMSP into dimethyl sulfide (DMS) and acrylic acid. It is found in many of the Ulvales and several species of Codium in the northeastern Pacific and Australasian regions. Many green algae such as *Ulva fenestrata* and *Enteromorpha linza* are avoided by urchins, which are deterred by DMS and acrylic acid in laboratory assays. However, these algae are often preferred foods of snails, which are deterred by DMS and acrylic acid. Snails may preferentially consume ulvoid green algae, despite being deterred by DMS and acrylic acid, because these algae contain relatively high nitrogen concentrations.

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### THE CHLOROPLAST GENOME OF THE TOXIC STRAMENOPILE *HETEROSIGMA AKASHIWO* (RAPHIIDOPHYCEAE)

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Algae live in an informationally dense environment. To ensure survival, many phytoplankton species respond to specific environmental signals by alternating between motile vegetative and non-motile resting life history phases. *Heterosigma akashiwo* (Raphidophyceae) can be induced to enter stasis by altering light, temperature and/or nutrient levels. In

the resting phase, this obligate photoautotroph can survive for months in cold, dark coastal sediments. To understand how *H. akashiwo* modifies chloroplast function as it moves between life history phases, we want to (a) identify the signal transduction mechanisms and (b) transcriptional processes in this organelle. Sequencing the chloroplast genome represents the first step in this endeavor. Electron microscopy, reassociation kinetic analysis and restriction analysis show the *Heterosigma* chloroplast DNA to be 154 kb. Chloroplast DNA recovered by Hoescht dye/CsCl centrifugation has been sheared to 700 bp fragments, cloned in pUC19 and sequenced according to standard protocols. All *Heterosigma* chloroplast genes lack introns, some genes overlap, others contain large repeats. At least one gene encodes large (90–100 amino acid) domains that are inexplicably inserted within highly conserved proteins and finally, some chloroplast genes encode proteins that have an extended 5' domain compared to homologues in other plastids. The *Heterosigma* genome has little colinearity with the stramenopile (*Odontella*), rhodophyte (*Cyanidium*, *Porphyra*), glaucophyte (*Cyanophora*), or cryptophyte (*Guillardia*) chloroplast DNAs that have been sequenced to date. Novel genes encoding signal transduction proteins have been identified.

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### PHYLOGENY OF THE CHLOROPHYCEAE: INFERENCES FROM THE CHLOROPLAST ATPB GENE

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Phylogenetic reconstruction using the 18S and 26S rDNA genes has proven successful for many chlorophycean lineages, but some nodes remain poorly resolved. The chloroplast-encoded *atpB* gene has been used to examine phylogenetic problems in several green algal lineages, including the basal streptophytes. However, data for other Chlorophyta are largely unavailable. The class Chlorophyceae, in particular, has not been examined (save for dense sampling among colonial and unicellular flagellates) using the *atpB* gene. An investigation of variability in the *atpB* gene for various chlorophycean exemplars was undertaken. Intergeneric distances (p) range from 0.08 (*Chlamydomonas reinhardtii* vs. *Volvox carteri*) to 0.24 (*Haematococcus lacustris* vs. *Chloromonas radiata*). Distance analysis also reveals high levels of divergence (as compared to the 18S and 26S rDNA data) for the family Sphaeropleaceae. Preliminary phylogenetic reconstructions fail to support a monophyletic Sphaeropleales, but this observation may be due to inadequate taxon sampling. The relative positions of other exemplars (e.g., *Chaetophora*, *Elakatothrix*, *Ascochloris*) are largely consistent with interpreta-

tions of 18S and 26S rDNA data, although the position of *Elakothrix* is not robust. These preliminary observations suggest that the *atpB* gene will provide a powerful complement to current phylogenetic assessments of the Chlorophyceae using 18S and 26S rDNA data. Supported by NSF DEB 9726588 and DEB 0129030.

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### ALGAL MONITORING STUDIES ON REMOTE TROPICAL PACIFIC REEFS

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Coral reefs of US-held islands in the central Pacific Ocean are among the most pristine in the world and represent over 93% of the reef systems under United States jurisdiction. The remote location of many islands has limited past algal research, resulting in incomplete understanding of species diversity, quantity, and ecology. Starting in 2000, the Coral Reef Ecosystem Investigation (CREI) began rapid ecological assessments on many Pacific island reefs to monitor ecological changes in reef biota over time. During the past year, algal efforts have concentrated on the French Frigate Shoals (Northwestern Hawaiian Islands) where we have increased the number of algal species reported by 1000%. Additionally, species new to science, including *Acrosymphyton brainardii* and *Scinaia huismanii*, have been described. Quantitative field sampling using a photoquadrat method is revealing species of the green algae *Halimeda* and *Microdictyon* to be ecological dominants in many areas during late summer/early autumn. Preliminary analyses with Primer software show species composition and abundance of all benthic organisms to differ significantly between most field sites sampled. Additional benthic habitat mapping of Pacific island reefs by CREI researchers is breaking the long-held paradigm that macroalgal cover is minimal in healthy tropical reef systems. Videotape analyses of benthic communities often find over 50% algal cover from 1 to 20 meter depths in many locations. Common ratios of macroalgae, turf algae, and crustose coralline algae to corals, other benthic organisms and substrate types on US Pacific reefs are being calculated for the first time.

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### CELL WALL PROTEOMICS OF THE GREEN ALGA *HAEMATOCOCCUS PLUVIALIS* (CHLOROPHYCEAE)

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The green microalga, *Haematococcus pluvialis*, which can synthesize and accumulate large amounts of the ketocarotenoid astaxanthin, undergoes profound changes in cell wall composition and architecture during the cell cycle and in response to environmental stress. In this study, cell wall proteins (CWPs) of *H. pluvialis* were systematically analyzed by SDS-PAGE coupled with peptide mass fingerprinting (PMF) and sequence-database searching. In total, 163 protein bands were analyzed, which resulted in positive identification of 106 protein orthologues. The highly complex and dynamic composition of CWPs are manifested by the fact that the majority of identified CWPs are differentially expressed during specific stages of the cell cycle along with a number of common wall-associated 'housekeeping' proteins. The detection of cellulose synthase orthologue in the vegetative cells suggested that the biosynthesis of cellulose occurred during primary wall formation. A transient accumulation of a putative cytokinin oxidase at the early stage of encystment pointed to a possible role in cytokinin degradation while facilitating secondary wall formation. This work represents the first attempt to use a proteomic approach to investigate CWPs of microalgae. The reference protein map constructed and specific protein markers obtained from this study provides a framework for future characterization of the expression and physiological functions of the putative proteins involved in the biogenesis and modifications of the cell wall of *Haematococcus* and other related organisms.

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### CYANOBACTERIA, TAXONOMY AND AQUATIC ODOUR: WITHIN- AND AMONG-SPECIES DIFFERENCES RE-EXAMINED

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Aquatic taste and odour (T/O) is most often associated with musty/earthy volatile organic compounds (VOCs) produced by cyanobacteria, the most-studied taxa in T/O research. In fact although cyanobacteria represent over 200,000 described morphological species, relatively few (< characteristics. non-robust apparently on based classification taxonomic to approach morphological traditional the re-examine need underline also results These management and

prediction odour for samples, field in taxa these of identification implications profound has This flasks. culture treatments same within even variable, highly fact are – coiling trichome shape, size akinete cell species this define currently which criteria key Several geosmin. compound production capita per morphology, differences intra-strain inter-marked show isolates ranges, natural representative levels over varied nutrients) temperature, light, (e.g. parameters major where conditions under variation production<sup>n</sup> VOC vitro their compares CA, Lake Castaic Ontario from lemmermanii *Anabaena* study a presents paper protocols. iv) culture; long-term changes physiological undergone have or unconfirmed, is original whose strains, collection studies; iii) environment; growth with morphology; ii) misidentification; i) from: stem may ambiguity. Much treatment proactive development confounds Clearly, trait. robust not that suggest some sources, O T as confirmed been 0.025%)> .

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### WETLAND DIATOM ASSEMBLAGES AS INDICATORS OF FLOODING INFLUENCE AND SURROUNDING LANDUSE IN THE WILLAMETTE VALLEY, OR

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The objective of this study was to examine the influences of the adjacent river and surrounding landuse on wetland diatom distributional patterns. Diatoms were identified in surface sediment samples from 35 riverine-impounded wetlands within the Willamette Valley, OR. A total of 221 species were identified and no single species dominated the assemblage at all sites. Diatom richness was high throughout the area (median 71, range 66–75). The Araphidineae:Centrales index, a measure of periphytic to planktonic species ratio, was lowest at sites within the river's annual floodplain zone. A low A:C index is to be expected at wetlands that receive inputs of river planktonic species through regular flooding by adjacent large rivers. Surrounding landuse can confound the influence of riverine flooding on wetland diatom assemblages by influencing water quality. Relative abundance of the two most common species, *Aulacoseira crenulata* and *Fragilaria capusina* related negatively to % agricultural landuse surrounding the wetland. These results indicate that riverine wetland diatom assemblages may be influenced by flooding from adjacent rivers and surrounding landuse and therefore wetland sediment diatoms might serve as useful indicators of both historical environmental changes in nearby large rivers and surrounding watersheds.

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### LIGHT-INDUCED GROWTH OF WINTER PHYTOPLANKTON COLLECTED FROM THE BENTHIC BOUNDARY LAYER IN COASTAL OREGON WATERS

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It has been speculated that overwintering phytoplankton in the benthic boundary layer (BBL) are seed stocks for spring blooms in coastal upwelling systems. Experiments were conducted in Jan/Feb 2003 to test the hypothesis that phytoplankton in the BBL are light limited. Water collected from the BBL at four sites across the northern Oregon shelf was incubated at in situ temperature and nutrient concentrations ( $\sim 1 \mu\text{M}$  phosphate,  $10 \mu\text{M}$  nitrate,  $15 \mu\text{M}$  silicate) and exposed to two different daylengths and four light levels. The daylengths were 9 h (winter) and 12 h (early spring), and the light levels (in  $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ) were 0 (Control), 20 (L), 70 (M), and 160 (H). Light-induced growth was observed in all four incubations. Exponential growth was reached in the 12 H, 12 M, and 9 H treatments after 4 to 6 days, and stationary growth was reached after 5 to 10 days. Maximum in vivo fluorescence was obtained in the 12 H, 12 M, and 9 H treatments, while maximum particulate organic carbon (POC) was obtained in the 12 H treatment. In vivo fluorescence increased in the L light treatments relative to the control, but POC did not. Blooms were terminated by nitrate or silicate depletion. These results demonstrate that growth of winter phytoplankton off Oregon is primarily light-limited, and that input of light of sufficient intensity and constancy (i.e. water column stabilization) can lead to blooms on time scales typical of upwelling blooms (<1 week).

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### FLEXIBLE ELEMENTAL STOICHIOMETRY IN TRICHODESMIUM IMS101: IMPLICATIONS FOR BUOYANCY-MEDIATED VERTICAL MIGRATION

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The cyanobacterium *Trichodesmium* is considered the most abundant and active nitrogen fixing plankton genus in tropical and subtropical marine waters. In the northern subtropical gyres of the Pacific and Atlantic Oceans, this organism is notable as a source of new production and as a potential biological shuttle for phosphorus to surface waters. We have conducted recent laboratory experiments revealing the remark-

able stoichiometric flexibility exhibited by this diazotroph. These results indicate that *Trichodesmium* spp. are capable of viable growth with carbon to phosphorus ratios approximately 16 times Redfield stoichiometry. Such P-sparing is clearly an adaptation to the oligotrophic environments from which these cultures were isolated. Building on this research, additional work was performed to determine the maximal temporal scale of vertical migration and the implicit dark period physiology. An experiment was designed to assess the physiological changes that would occur as cells of varying growth stages migrated below the euphotic zone into more phosphorus replete waters. At different stages of growth sub-samples of phosphorus replete or phosphorus limited cells were transferred to dark conditions. For each dark bottle, chl-a, particulate carbon, phosphorus and nitrogen, trichome count and fluorescence parameters were measured daily. Our experimental results indicate that the maximum duration of dark period survival (and hence vertical migration) is approximately three days during which period cells were able to fully recover from light deprivation and resume typical growth rates. The knowledge of *Trichodesmium* bioenergetics gained from this research will serve to further constrain the potential role of *Trichodesmium* spp. in biogeochemical cycling.

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**CHALLENGES IN PREVENTING, ERADICATING, AND CONTROLLING INVASIVE SEAWEEDS: THE SOUTHERN CALIFORNIA INVASION OF *CAULERPA TAXIFOLIA* AS A MODEL**

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Invasions of marine communities by seaweeds are increasing globally. Vectors for introductions are primarily aquaculture and the aquarium trade. Attention to these vectors has been minimal, and few, if any, regulations exist to prevent seaweed introductions. Challenges in preventing, eradicating, and controlling invasive seaweeds will be discussed using the invasion of *Caulerpa taxifolia* in southern California as a model.

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**PHYLOGENETIC RELATIONSHIPS WITHIN *CENTROCERAS KUETZING* (CERAMIACEAE, RHODOPHYTA)**

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*Centroceras clavulatum* (C. Agardh) Montagne (1846) is a widely distributed species worldwide, originally described from Callao, Peru. Populations referred to in the literature as *C. clavulatum* were investigated on the basis of rbcL sequence analysis and comparative morphology. Four well-supported clades were identified. RbcL sequence divergence within clades was <0.8, between clades 1.1–8.1%. We conclude that *C. clavulatum* may encompass at least three additional species that may have been placed under its synonymy. The four well-supported clades under study are distinct from *Centroceras internitens* Gallagher & Humm and include, respectively, representative specimens with the following distribution: (1) California, Gulf of California, Korea, Japan, (2) E Florida, Texas, (3) California, and (4) W and E Florida. Studies are underway to assign a correct species name to each of the clades under study.

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**TOWARD AN OPTICAL BIOGEOGRAPHY OF THE OCEANS**

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Remote sensing of ocean color has revolutionized our ability to understand the processes leading to the observed distribution of different taxa in marine waters. Many scientists in the remote sensing and optics community are working toward retrieval of species distributions using ocean color measurements to derive the concentration of recognized chemotaxonomic markers. In this talk, I work toward an optical biogeography of the ocean by viewing the optical environment as a selection regime that creates biogeographic boundaries or “optical fences” defining the distribution of taxa with different light harvesting systems and/or different physiologies. Working primarily with data from a wide range of tropical, subtropical, and warm temperate coastal margins, I show that there is a close association between the distribution of different spectral forms of PE-containing picocyanobacteria and the optical properties of the water masses in which they are found. This pattern also appears to be reflected in the distribution some dinoflagellate taxa, indicating that the optical environment encompasses a range of key niche parameters that, in turn, determine the biogeographic distribution of species.

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**NEW DISCOVERIES REGARDING THE BENTHIC MARINE ALGAL FLORA OF THE SULTANATE OF OMAN**

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Intensive littoral and sublittoral collecting on the monsoon-impacted rocky shores of southern Oman, made at the end of the summertime monsoon (Sept. 2000 and Sept. 2001), has produced a remarkably high number of undescribed taxa and new records for this region of the Northern Arabian Sea. Taxa to be discussed in this presentation include: a new species of *Centroceras* that reaches 60 cm in height, is heavily corticated, has distinctively secund branching, and occurs well into the 'supralittoral fringe'; a new species of *Jolyina*, which is often branched, is not as long or thick as the more common *J. laminarioides*, has ascocysts but lacks hair tufts, and also occurs in the supralittoral; a *Turbinaria* (*T. foliosa*) that has the least modified blades known in the genus; a remarkably robust and erect species of *Leveillea*; and an undescribed *Gelidium*, which has previously passed under the name of '*Suhria vittata*'. A complicated picture has emerged that shows this flora to have biogeographical affinities with pantropical and subtropical regions, the tropical-subtropical Indo-Pacific, South Africa, temperate Europe and the Mediterranean, Japan, as well as revealing components that are characteristic of the Arabian Sea monsoon season and apparent endemics restricted to Oman. This research (the Algal Biodiversity Project of Oman) has been funded by the British Government's Darwin's Initiative grant for the 'Survival of Species' and managed by HTS Development, Ltd., of the U.K.

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#### MOLECULAR SYSTEMATICS OF THE GREEN ALGAL ORDERS ULOTRICHALES AND ULVALES BASED ON 18S RDNA SEQUENCES

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The traditional use of thallus morphology to define lineages within the Ulvophyceae proved problematic because parallelism and convergence obscured natural relationships. The advent of transmission electron microscopy led to a re-evaluation of groups on the basis of zoid characters. Within TEM-defined lineages, the Ulotrichales and Ulvales were considered closely related orders, and molecular evidence has substantiated this hypothesis. In our molecular systematic studies of ulvophyceous green algae, we have broadened taxon sampling among these two orders. We show that the Ulvales and Ulotrichales are indeed monophyletic orders. However, the phylogenetic placement of species in the genera *Bolbocoleon*, *Ctenocladus*, *Phaeophila*, *Pseudendoclonium* and *Acroblaste* indicates that one or more additional lineages, placed between Ulvales and Ulotrichales in gene sequence trees, must be recognized. These species may constitute a separate order (Ctenocladales) consisting of multiple families. Our findings

will be compared with taxonomic concepts based on morphological criteria.

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#### MOLECULAR SYSTEMATICS OF THE ULVELLACEAE (ULVALES, ULVOPHYCEAE) INFERRED FROM NUCLEAR AND CHLOROPLAST DNA SEQUENCES

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The diversity and phylogeny of endophytic, micro-filamentous green algae in the family Ulvellaceae (Chlorophyta: Ulvophyceae) are poorly understood. Members of this family have suffered a long history of confusing taxonomy, resulting from phenotypic plasticity and few stable morphological characters. Using DNA sequence data from two molecular markers, the plastid encoded elongation factor TU (*tufA*) and the nuclear encoded small subunit ribosomal DNA (18S rDNA), we tested taxonomic concepts in Ulvellaceae. Concordance between plastid and nuclear phylogenies revealed the following: 1) Ochlochaete is distinct from Acrochaete and may represent a separate family, 2) Ochlochaete is not monotypic, consisting of at least two genetically distinct species, 3) the tribe Ulvelleae, separated from the Acrochaeteae on the basis of radial (versus linear) germination and bifurcate apical cells, is not supported as one of the members, *Pringsheimiella scutata* groups with a suite of Acrochaete species and 4) the Ochlochaete clade is the proximal out-group for the Ulvaceae. These findings will be presented in the light of morphological characters that are currently used to define species and genera in the Ulvellaceae.

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#### CONJUGATION PROCESS OF A FRESHWATER GREEN ALGA, *SPIROGYRA VARIANS* (ZYGNE-MATALES, CHAROPHYCEAE) EXAMINED BY FITC-LECTINS

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The conjugation processes of filamentous freshwater green alga *Spirogyra varians* were examined by the use of FITC-lectins. Conjugation comprised of five steps: 1) array with adjacent filaments, 2) formation of conjugation protrusion (papilla), 3) fusion of the protrusions, 4) formation of conjugation tube, and 5) formation of zygotes. Three lectins, ConA, RCA and UEA, showed considerable labeling during the progression of conjugation. FITC-ConA labeled the

surfaces of filaments throughout the whole conjugation processes. No labeling of FITC-RCA was detected on the surface of vegetative filaments. FITC-RCA labeling was observed at the conjugation protrusions only after the papilla formation. Strong labeling continued until formation of zygotes even in hollow area between the conjugation tube. The labeling decreased gradually over time and disappeared when zygotes were formed. FITC-UEA showed similar labeling pattern with FITC-RCA except that the labeling did not disappear even after zygote formation. Inhibition experiments using D-galactose, L-fucose and D-mannose, which are complementary carbohydrates for the above lectins, showed considerable decrease of conjugation (<50% vs. 83% in control). Hamagglutination experiment using crude extract of *Spirogyra varians* revealed existence of lectins specific for the above carbohydrates. These results suggested that the lectin-carbohydrate recognition system might be involved in the conjugation of *Spirogyra varians*.

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### RELATIONSHIPS BETWEEN WATER AND SEDIMENT CHARACTERISTICS AND BENTHIC GREEN MACROALGAL ABUNDANCE IN YAQUINA BAY, OREGON: 1999–2002

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“Green tides” or blooms of ulvoid green algae are frequent in Yaquina Bay estuary on the central Oregon coast, USA. Measurements of their biomass were made from late spring to early winter in 1999 at six intertidal sites in the estuary, and were continued through the winter of 2002 at two sites that showed the greatest accumulation. The dominant blooming species were the *Enteromorpha linza* complex, *Ulva fenestrata*, *E. flexuosa*, and *E. intestinalis*. Red and brown algal abundance was negligible. Nutrients in the central channel were monitored, along with incident light and water column absorbance, and sediment temperature. The abundance of benthic amphipods was negatively correlated with the concentration of dissolved sulfides in porewater. However, dissolved sulfide levels were not correlated with green macroalgae biomass. Additional correlations were sought between the variables measured in an effort to suggest or negate major cause and effect relationships. This research is relevant to discerning the effects of eutrophication as a stressor on estuarine

processes and could contribute toward an understanding of anthropogenic impacts on biological communities in coastal ecosystems.

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### TWO LINEAGES WITHIN *GRIFFITHSIA* (CERAMIACEAE, RHODOPHYTA) BASED ON PLASTID PROTEIN-CODING PSAA, PSBA AND RBCL GENES

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The ceramiaceous red algal genus *Griffithsia* C. Agardh includes about 27 species that are distributed along tropical to temperate coasts. Although *Griffithsia* has been subdivided into four groups based on morphology, there are no attempts on molecular phylogeny of the genus. Sequences of *psaA*, *psbA*, and *rbcL*, protein-coding genes in plastid, were analyzed for nine samples of seven *Griffithsia* species, 11 samples of four putative relatives such as *Anotrichium Nägeli* and *Halurus Kützing*, and three outgroup taxa. Although sequence divergences for *psaA* and *rbcL* were higher than those for *psbA*, the saturation plots for each of *psaA*, *psbA*, and *rbcL* data showed no evidence of saturation at 1st, 2nd, and 3rd codon position. Partition homogeneity test also indicated that *psaA*, *psbA*, and *rbcL* sequences did not result in significantly incongruent trees. Analyses of separate and concatenated data sets indicated that *Griffithsia* formed two well-supported clades: one lineage including *C. corallinoides*, *G. pacifica*, and *G. tomo-yamadae*, and the other lineage having *G. antarctica*, *G. japonica*, *G. teges*, and *G. traversii*. Our study indicates that *psaA* and *psbA* genes as well as *rbcL* are suitable tools for phylogenetic investigations of the genus *Griffithsia*.

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### EXPERIMENTAL HARVESTS OF FIVE SPECIES OF MACROALGAE ALONG THE OREGON COAST

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Macroalgae are the basis of a major global industry. Their nutritional and chemical values have brought strong economic pressure for commercial harvest permits to the State of Oregon, USA. Historically, Oregon has denied these requests because of a lack of understanding of harvest effects on the resource. Three species of phaeophytes (*Laminaria setchellii*, *Alaria marginata*, *Fucus gardneri*) and two rhodophytes

(*Mastocarpus papillatus*, *Mazzaella splendens*) were subjected to various treatments to determine their ability to recover from harvest. Three year experiments were designed to compare the effects of harvesting during different seasons, different amounts, and different methods. Permanent transects and plots were placed in three study sites along the Oregon coast. Here we present the results from the first growing season. Only *A. marginata* was found to regain pre-harvest biomass within one growing season; all species will be monitored for regrowth and resettlement for the next two growing seasons to provide a basis for the management of the potential economic resource.

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#### THE ENVIRONMENTAL IMPACTS OF HARVESTING BEACH-CAST SEAWEEDS

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Until recently the commercial collection of beach-cast seaweeds in New Zealand was prohibited but the legislation has recently been amended to allow permitting of this activity. This review collates existing information on the role of beach-cast seaweed in coastal ecosystems to describe the nature and extent of the effects that commercial removals of beach cast seaweed may have on the marine environment. It outlines the amount of beach-cast seaweed available for harvest in New Zealand and the fate of seaweed when not collected; reviewing current information on the importance of beach-cast seaweeds and its inhabitants on: feeding and nesting shorebirds, and nutritional contribution of seaweed inhabitants to nearshore coastal ecosystems when seaweed is washed back into the sea. It also identifies key research gaps related to any environmental impacts that the removal of beach cast seaweed might have.

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#### EXPRESSED GENE ARRAYS IN THREE MARINE DINOFLAGELLATES WITH CONTRASTING TROPHIC MODES ANALYZED VIA A SMALL-SCALE EST SEQUENCING

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In an attempt to determine the common and unique the genes functioning for different trophic modes in dinoflagellates, cDNA libraries were constructed for *Prorocentrum minimum* (photoautotrophic), *Karlodinium micrum* (mixotrophic), and *Pfiesteria piscicida* (kleptoplastic heterotrophic). Randomly selected

100–350 clones were sequenced for each species. Results revealed a small number of genes transcribed in all three species (basic nuclear proteins, ribosomal proteins). There appeared to be a suite of unique genes in each species. In *P. piscicida* that was fed *Rhodomonas* sp., the array of expressed genes differed clearly among different growth conditions. The cDNA library prepared for samples collected 12 h after feeding contained more photosynthetic genes (light harvesting complex proteins) and ribosomal proteins, while growth-related genes were expressed more abundantly at 24 hours after feeding. At 48 hours when growth rate was decreased dramatically, the most abundant gene transcripts found were those involved in metabolism (mt cob, cox1 and cox3). In addition, several genes (ras, rac, chaperonin) appeared to be identical at amino acid level among the two heterotrophic or mixotrophic dinoflagellates (*P. piscicida* and *K. micrum*) and a prey alga (*Rhodomonas* sp.), although the likelihood of contamination was small. These results suggest that some of the dinoflagellate genes may be originated through horizontal transfer from historical prey species and RT-PCR measurements indicate that these genes maybe highly expressed in dinoflagellates.

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#### PHYTATE UTILIZATION BY TETRAHYMENA

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Phytate, myo-inositol hexakisphosphate, is the storage form of phosphate in seeds and grains and a major form of environmental phosphate loading from fertilizer inputs and agricultural runoff. Little is known about the effects of phytate on heterotrophic protozoa. Members of the genus *Tetrahymena* were chosen as the model organisms for this study because of their wide geographic distribution and adaptability. We found that *Tetrahymena* can use phytate as their sole phosphate source and that growth is comparable to that for cells grown in media containing orthophosphate. The internal phytate concentration increases dramatically during the logarithmic phase of growth and then decreases during the stationary phase. These internal levels in *T. vorax* are as much as 10-fold greater than those for cells grown in phosphate without phytate, suggesting diet as a main source of accumulation. In *T. thermophila*, the internal phytate level also decreases during stationary phase, but the levels during both logarithmic and stationary phase of growth are similar whether cells are grown in phytate or phosphate. *Tetrahymena thermophila* possesses several nonspecific acid phosphatases capable of using phytate as a substrate including one associated with the pellicle. We are examining the properties of this phytase.