

**ABSTRACTS OF PAPERS SCHEDULED FOR THE  
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Abstracts of papers presented at this year's meeting are published as a supplement to the June issue of the *Journal of Phycology* and mailed to members.

1

### **AN ANTARCTIC FEEDING TRIANGLE: CHEMICAL DEFENSE, PHYSICAL DEFENSE, AND MUTUALISM IN MACROALGAE, URCHINS, AND ANEMONES**

Amsler, C. D.<sup>1</sup>, McClintock, J. B.<sup>1</sup> & Baker, B. J.<sup>2</sup>

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We document a unique relationship between red macroalgae (*Phyllophora antarctica* and *Iridaea cordata*), the sea urchin *Sterechinus neumayeri*, and the sea anemone *Isotelia antarctica*. We have shown that both macroalgal species are chemically defended against herbivory by *S. neumayeri*. This is supported by studies showing macroalgae to be only minor components of the urchin gut contents. However, urchins prefer to cover themselves with these macroalgae. At sites in nature where macroalgal drift is present, over 95% of the urchins use macroalgae as cover and the vast majority of available drift is being held by them. When presented with equal amounts of macroalgae and other cover materials in the laboratory, "naive" urchins collected at sites without macroalgae have a strong preference for macroalgae. The major predator on sea urchins in this system is the sea anemone *I. antarctica*. The presence of algal cover on the urchins significantly increases the likelihood of escape from *I. antarctica* during a predation event because the anemones' tentacles attach to the algae, which the urchins then release as they escape. This defense is physical, not chemical, as thalli from which defensive chemicals have been extracted are equally protective. The macroalgae benefit from this unique relationship because large numbers of fertile drift plants are retained in the photic zone by these dominant, circumpolar urchins and, presumably, continue to release spores and contribute to the gene pool rather than being washed onto shore or into deep water.

2

### **PHYLOGENY OF CHRYSOPHYCEAE USING 18S rDNA AND rbcL SEQUENCES, WITH COMMENTS ON ULTRASTRUCTURE AND CLASSIFICATION**

Andersen, R. A.<sup>1</sup>, Potter, D.<sup>2</sup>, Daughjerg, N.<sup>3</sup> & Bailey, J. C.<sup>1</sup>

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The 18S rRNA and rbcL genes were determined for representative members of the Chrysophyceae. Phylogenetic trees from parsimony and distance analyses are largely congruent. However, this molecular phylogeny is starkly at odds with the traditional Pascher/Bourrelly/Starmach scheme of classification for the class. For example, *Chromulina nebulosa* (Chromulinaceae, Chromulinales) is most closely related to *Chrysamoeba pyrenoidosa* (Chrysamoebaceae, Chrysamoebales). Also, *Cyclonexis annularis* (Ochromonadaceae, Ochromonadales) is most closely related to *Phaeoplaca thallosa* (Phaeoplacaceae, Phaeoplacales). Some genera, such as *Dinobryon* and *Epipyxis*, have been considered to be so closely related that some workers have placed them in a single genus; molecular data suggest they are distantly related. Ultrastructural data, where available, generally support the molecular phylogenies. For example, *Hibberdia* and *Chromophyton* have very similar flagellar apparatuses, and they are sister taxa in molecular analyses. Conversely, *Lagynion* is reported to have a flagellar apparatus similar to *Hibberdia*, but these genera are inferred to be distantly related in our analyses of molecular data. The recent removal of several groups from the Chrysophyceae [e.g., Chrysomeridiophyceae, Dictyocophyceae (including pedinellids and *Rhizochromulina*), Haptophyceae, Pelagophyceae (including Sarcinochrysidales), Phaeothamnio-phyceae, and Synurophyceae] and the recent addition of ultrastructural and molecular data indicate that the classification scheme for the Chrysophyceae should be re-evaluated.

3

### **IDENTIFICATION OF GENES EXPRESSED SPECIFICALLY WHEN THE CENTRIC DIATOM THALASSIOSIRA WEISSFLOGII UNDERGOES SEXUAL REPRODUCTION**

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An intriguing aspect of the diatom life cycle is that each mitotic division creates one daughter cell that is the same size as the parent cell and one daughter cell that is slightly smaller. Thus, the mean cell size of most diatom populations decreases over successive generations. A common manner of escaping this trend

of diminishing cell size is through sexual reproduction. Importantly, only those cells with diameters below a minimum size can be induced to undergo gametogenesis and subsequent zygote (auxospore) formation. The newly formed auxospore expands to generate a post-auxospore cell which is much larger than either parent cell and is once again resistant to sexual induction cues. As a first step towards understanding the molecular basis for this coupling between cell size and responsiveness to sexual triggers, genes expressed specifically during gametogenesis have been identified. Two clonal cell lines of the diatom *Thalassiosira weissflogii* have been isolated; cells from one line have relatively small cell diameters and can be induced to undergo gametogenesis whereas cells from the other line have relatively large cell diameters and are immune to the same induction cues. A PCR-based technique has been used to compare the genes expressed by these two cell lines upon exposure to an induction trigger. A subtraction library of partial cDNAs expressed only in those small cells undergoing sexual reproduction has been created. Eight distinct cDNAs have thus far been identified and are now being sequenced. Possible roles played by these genes during gametogenesis will be discussed.

#### 4

### **ELECTRON MICROSCOPY MAY REVEAL STRUCTURE OF DOCOSAHEXAENOIC ACID-RICH OIL WITHIN THE STRAMENOPILE, *SCHIZOCHYTRIUM* SP.**

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As a thraustochytrid, *Schizochytrium* sp. is a unicellular algae-like microorganism closely related to the heterokont algae. *Schizochytrium* sp. is utilized for the commercial production of docosahexaenoic acid (DHA)-rich oil and biomass for use as food and feed ingredients such as dietary supplements and DHA for infant formulae. Electron microscopic analysis of *Schizochytrium* sp. employing cell sample preparation by high pressure freeze substitution revealed possible secondary and tertiary semi-crystalline/crystalline structures of the triacylglycerols within the microbial oil bodies. A fine secondary structure consisting of alternating light and dark-staining bands was observed inside the oil bodies. The dark bands were  $28.78 + 1.35 \text{ \AA}$  in width and light bands were  $21.56 + 1.35 \text{ \AA}$  in width. The tertiary (three-dimensional) structure appears to be a multi-layered ribbon-like structure which

is coiled and interlaced within the oil body. Confirmatory freeze-fracture photomicrographs of *Schizochytrium* sp. exhibited fracture planes with terraces averaging  $52.18 + 6.8 \text{ \AA}$  in height and correspond to the combined width of two halves of two light bands and one dark band observed in the high pressure freeze substitution photomicrographs. The results suggest the triacylglycerols within *Schizochytrium* sp. oil bodies may be organized in a triple chain length structure and a model for this structure is proposed. These results are also compared to oil bodies in electron micrographs of several comparable oil-producing species of fungi and algae with known fatty acid compositions employing the same high-pressure freeze substitution technique.

#### 5

### **COMPARISON OF *SPIRULINA* GROWTH IN A TUBULAR BIOREACTOR AND AN OPEN RACEWAY POND**

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Outdoor mass culture of *Spirulina* has traditionally been carried out in open raceway ponds and there are numerous commercial operations that utilize this method. However, many publications have demonstrated the advantages provided by enclosed culture systems, and work in this area has led to the development of several prototypes. We report initial results using a small scale enclosed tubular reactor system recently installed at our facility in Lakeview, California. This tubular system which utilizes computer control to continuously monitor culture parameters will be described, as well as data detailing the growth and productivity of enclosed and open systems operating under identical environmental conditions.

#### 6

### **GENETIC VARIABILITY AMONG ISOLATES OF TWO SPECIES OF BROWN TIDE ALGAE**

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Episodic blooms of the picoplanktonic "brown tide" algae *Aureococcus anophagefferens* and *Aureoumbra lagunensis* (Pelagophyceae) along the coasts of the northeastern US and in the Laguna Madre basin of Texas, respectively, have disrupted marine ecosystems and negatively impacted fisheries. Although

physiological and taxonomic studies of these algae have been published, little is known of their genetic diversity or population biology. We used molecular biological tools to determine the level of genetic variability within geographically and temporally defined isolates of *Aureococcus* and *Aureoumbo*. Complete nucleotide sequences for the nuclear 18S rRNA gene, the plastid-encoded *rbcL* gene, as well as the RuBisCO spacer region have been determined for four isolates of *Aureococcus*. Sequences for each of these three regions of DNA are identical in all *Aureococcus* isolates. The 18S rRNA sequences determined in our laboratory for *Aureococcus* do, however, differ from a previously published sequence for this organism. Both *rbcL* and RuBisCO spacer sequences have also been deduced for three isolates of *Aureoumbo*. Sequences for these DNA regions were found to be identical in each of the three *Aureoumbo* isolates and are different from those for *Aureococcus*. In contrast to the sequence data where variability has yet to be found within species, genetic polymorphisms among the three *Aureoumbo* isolates have been revealed by RAPD markers and this technique is now being applied to *Aureococcus* isolates as well. Results of analyses of new isolates as well as DNA fingerprinting studies will be presented.

## 7

### THE PHAEOTHAMNIOPHYCEAE: A NEW CLASS OF CHROMOPHYTE ALGAE

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A new algal class, the Phaeothamniophyceae, is described on the basis of ultrastructural information, photosynthetic pigment data, and analysis of plastid *rbcL* and nuclear 18S rRNA gene sequences. Included in the class are, among others, three genera formerly classified in the Chrysophyceae (*Phaeschizochlamys*, *Phaeothamnion* and *Stichogloea*) and one traditionally assigned to the Xanthophyceae (*Pleurochloridella*). Ultra- structural studies indicate that: 1) cell walls are present in most, if not all, species, 2) zoospore basal bodies form an angle of ca. 145 degrees, 3) a multi-gyred transitional helix is present, 4) a chloroplast girdle lamella is present, 5) the chloroplast genophore is ring-shaped, and 6) numerous electron opaque vesicles are present consistently at the cell periphery. The chemical composition of the photosynthetic storage product and sexual reproduction are unknown. In addition to chlorophylls *a* and *c*, HPLC analysis revealed the

presence of fucoxanthin, diadinoxanthin, diatoxanthin, heteroxanthin and  $\beta,\beta$ -carotene. The combination of fucoxanthin and heteroxanthin are not found in any other chromophyte algal class. Phylogenetic analysis of *rbcL* and 18S rRNA gene sequences will be presented; these data position the Phaeothamniophyceae within a clade that also includes the Phaeophyceae and Xanthophyceae. Other genera that are best classified in the Phaeothamniophyceae include *Chrysoclonium*, *Phaeobotrys*, *Phaeogloea*, *Sphaeridlothrix*, *Tetrachrysis*, *Tetrasporopsis* and, perhaps, *Chrysapion*, *Koinopodion*, *Selenophaea*, and *Tetrapion*.

## 8

### CHANGES IN INTRACELLULAR C:N RATIO DURING GROWTH OF MARINE MICROALGAE UNDER BATCH CULTURES

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Under batch cultures the chemical composition of microalgae may vary largely, as a complex response to experimental conditions. The C and N contents of ten marine microalgae were studied at mid-log, late-log and stationary growth phases of cultures. Species were cultured with Conway medium, with and without addition of bubbling air. C and N contents show different trends of variation during growth of species, but both elements attained higher percentages in cultures with bubbling air. Except for *Prorocentrum minimum*, great changes in C:N ratio occurred during growth of microalgae. For each species values of C:N ratio obtained with the two treatments were similar at mid-log and late-log growth phases, varying from 4.36 (*Synechococcus subsalsus*) to 7.77 (*Tetraselmis gracilis*), but C:N values were higher at the stationary growth phase of cultures that grew with bubbling air, ranging from 8.82 (*Hillea* sp.) to 15.7 (*Nannochloropsis oculata*). Cultures under bubbling air always showed small amounts of intracellular inorganic nitrogen (IIN), measured as nitrate, nitrite and ammonium. On the other hand, during all experimental period cultures that grew without bubbling air showed lower total protein per cell and a greater content of IIN, mainly *Skeletonema costatum*, *Chlorella minutissima* and *Isochrysis galbana*. For cultures carried out under high nitrogen concentrations in culture medium, the lack of suitable carbon supply generates two important

effects: the occurrence of large pools of IIN within cells and a slower production and accumulation of protein.

## 9

### **EFFECTS OF PAR AND UV-IRRADIANCE ON PHOTOACCLIMATION RESPONSES IN A TROPICAL RED ALGA.**

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Analysis of absorbance spectra of the red alga, *Ahnfeltiopsis concinna*, revealed the capacity of tissues from canopy and understory to respond to changes in the intensity of PAR. No differences in photoacclimation responses were observed for experiments performed with *A. concinna* under PAR-only, PAR+ UV-A, and PAR+ UV-A+ UV-B. *In situ*, photoacclimation capacity was manifested as differences in pigmentation for tissues from canopy to understory microsites of this dense turf. This pigment adjustment to PAR is dynamic and recovery from irradiance stress induced by canopy removal was complete within 25 days. *In vivo* absorbance spectra allowed simultaneous observation of multiple pigment pools and monitoring photoacclimation changes over days in this alga. Insight into the cascade of photoacclimation events was revealed by concurrent measurement of pigments and photosynthetic performance. Overall, acclimation from a sun to shade or shade to sun acclimation states proceeded faster for degradative than for synthetic processes. Specifically, phycobilin degradation in acclimation to sun from shade was faster than phycobilin synthesis in tissues in acclimation from shade to sun. Phycoerythrin-specific absorbance changed to a greater extent than did shifts of compensation or saturation irradiance thresholds in acclimation from shade to sun. While compensation and saturation irradiances changed to a greater extent than phycoerythrin specific absorbance in acclimation from sun to shade. Lastly, carotenoid and putative mycosporine amino acid absorbances adjusted in response to changes in incident irradiance quantity regardless of irradiance quality. This suggests a similar signal transduction mechanism or other metabolic linkages in this photoacclimation mechanism.

## 10

### **SPIRULINA (ARTHROSPIRA) PRODUCTION: FROM THE RIFT VALLEY TO THE IMPERIAL VALLEY**

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Large, shallow soda lakes occupy the rift valleys in Ethiopia, Kenya and Tanzania and occur in southern Africa. Small but numerous soda lakes stretch northeast from Lake Chad and are scattered throughout the eastern and western rift valleys of eastern Africa. These soda lakes are recognized as among the world's most productive ecosystems. A feature of soda lakes throughout Africa is persistent, almost unialgal blooms of the cyanobacterium, *Spirulina platensis* (*Arthrospira platensis*). These lakes form the basis of our knowledge in the mass cultivation of *Spirulina*. Moreover, it was knowledge of the human use of *Spirulina* by the Kanebou tribes around the Lake Chad area, as far back as 1940, that stimulated research in the utilization of *Spirulina* as human food. *Spirulina* is now produced commercially in many countries. Current annual production of *Spirulina* is estimated at 2,000 metric tons. In this presentation, the productivity of *Spirulina* in outdoor cultures is discussed in relation to theoretical, and observed upper limits of production in these rift valley lakes, citing examples from the world's largest *Spirulina* farm located in the Imperial Valley of Southern California. The problems faced in outdoor, large-scale commercial production of *Spirulina* also will be discussed.

## 11

### **BIOMECHANICS AND SURVIVAL OF THE GIGARTINALES**

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Key to the survival of any sessile marine organism is the ability of the organism's mechanical design to meet the demands of its flow environment. Members of the order Gigartinales are no exception to this rule, and even thalli that inhabit "low flow" habitats encounter hydrodynamic forces that can challenge their strength of attachment. I will review the biomechanical principles that are relevant to macroalgae in flow, including the generation of hydrodynamic forces and the measurement of material properties (e.g., strength, strain, and stiffness). I will then discuss strategies for increasing thallus survival in flow, either by reducing hydrodynamic forces or increasing attachment strength. In particular, I will address the extent to which the flow environment can constrain thallus morphology and size. While the focus of this presentation will be on the Gigartinales, other examples from the macroalgal literature will be presented as well. In closing, I will

suggest areas for future research that would greatly contribute to our understanding of the mechanical design of this ecologically and economically important group.

## 12

### **THE PHYLOGENY OF GREEN ALGAL AND PLANT ACTINS**

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Green algae and land plants ('Viridiplantae') are sister groups which are phylogenetically subdivided into the Chlorophyta and the Streptophyta. The common ancestor of this lineage is believed to have been a member of the Prasinophyceae. We have sequenced the actin-encoding cDNAs from *Mesostigma viride*, *Scherffelia dubia* (Prasinophyceae), *Coleochaete scutata*, *Spirogyra* sp. (Charophyceae), and from two fern species, *Anemia phyllitidis* and *Ptilotum triquetrum* to address Viridiplantae evolution. Phylogenetic analyses show *Mesostigma* to be the earliest divergence within the streptophytes and provide evidence for a unicellular ancestor for this lineage. This is supported by two conserved actin introns (positions 20-3, 152-1) shared by *Mesostigma* and the embryophytes. The ferns contain multiple copy actin genes that form a monophyletic group positioned outside of the plant actin gene radiation. We identify three classes of actins in the Viridiplantae (Classes I, II, III). N-terminal "signature" sequence analyses support these groupings. The Class I actins include the single copy genes of the green algae and the multiple copy genes of the ferns. The Class II plant actins are often expressed in vegetative structures. The Class III plant actins trace their ancestry within the Class II sequences and contain members that are largely expressed in reproductive structures.

## 13

### **MULTIPLE GROUP I INTRONS IN THE SSU rRNA GENE OF THE CHLOROPHYCEAN ALGAE *SELENASTRUM CAPRICORNUTUM***

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A phylogenetic investigation of the green alga *Selenastrum capricornutum* utilizing the small subunit ribosomal RNA gene (SSU rRNA) has revealed numerous Group I (subset IC1) introns. *Selenastrum capricornutum* is an autosporic, uninucleate alga in the order Chlorellales. Ultrastructural examination of *S. capricornutum* for phylogenetic studies is limited because it lacks motile cells and the flagellar structures

used for comparisons in many green algae. Since proposed phylogenetic relationships in other green algal taxa based on ultrastructure have been supported by subsequent molecular investigations, a molecular phylogenetic examination of *S. capricornutum* was undertaken. During this study, six Group I introns were discovered within this gene. Previous studies have shown that numerous algal taxa contain Group I introns, and in some cases multiple introns have been found in a single ribosomal gene. Large numbers of group I introns are also observed in some fungal taxa. Group I introns are removed by folding of the intron into a conserved secondary structure that is required for correct excision. These introns are of evolutionary interest as a possible relict of an ancient RNA world. Three Group I introns of *S. capricornutum* are located at conserved algal intron insertion locations while the remaining three introns are found at novel positions. The SSU rRNA gene and the introns have been sequenced, and are reported here. Putative secondary structures for these introns are presented. Also, their primary nucleotide similarity to other Group IC1 introns found in algae, fungi, viruses, and other sources is investigated.

## 14

### **EARLY STAGES OF COMMUNITY DEVELOPMENT: BIOFILMS AND THEIR INFLUENCE ON SPORE SETTLEMENT OF *ENTEROMORPHA FLEXUOSA***

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The accretion of abiotic and biotic materials on substrates submerged in the marine environment results in the development of a complex biofilm. The characterization of early films from coastal areas on O'ahu, Hawai'i was evaluated both spatially and temporally via epifluorescence microscopy coupled with fluorescently-labeled lectins. Variation in the richness and evenness of lectin-specific glycoconjugates over 1, 3, 24, & 72 hr aged films demonstrated significant differences in early biofilms (1, 3, & 24 hr), but not between 24 & 72 hr films. Multi-species biofilms of different ages were observed to influence the process of spore settlement in *Enteromorpha flexuosa*, a common fouling green alga. On surfaces immersed for 0, 1, 3, 24, & 72 hr in Pearl Harbor, a positive correlation was detected between spore settlement of *E. flexuosa* and film age. Bacteria are an important component of biofilms and monospecific bacterial films used to evaluate settlement in a fouling invertebrate, *Hydroides*

*elegans*, were further tested with *E. flexuosa*. All monospecific films tested were found to have decreased spore settlement by the time the films were aged for 72 hrs. Films composed of a *Bacillus* sp. had significantly lower settlement in contrast to the *Vibrio* and *Pseudomonas* spp. Spores of *E. flexuosa* and the larvae of *H. elegans* were found to not respond similarly to cues present in two of the three bacterial films tested. This research provides an important link in understanding how biofilms can influence early stages of development of algal communities.

## 15

### **PRODUCTION OF ASTAXANTHIN-RICH HAEMATOCOCCUS: "FROM PETRI DISH TO SALMON DISH"**

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Astaxanthin (3,3'-diketo-4'-dihydroxy-beta-carotene) was first described in aquatic crustaceans as an oxidized form of beta-carotene, which gives the carapace of these animals its pinkish color. This pigment is used in aquaculture as a feed supplement in the production of salmon and shrimps, and as a colorant of egg yolk in the poultry industry. The green alga *Haematococcus* appears to be one of the most promising natural sources for this purpose. Though astaxanthin can be synthesized by other algae, bacteria and a few fungi, the amounts accumulated by *Haematococcus* surpass reported alternative sources. Astaxanthin accumulation in *Haematococcus* is accompanied by a remarkable morphological and biochemical "transformation" from green motile cells into inert red cysts and mediated by different environmental stress conditions. We have studied different aspects of this process and as an outcome of this research we have put forward a successful strategy for large-scale production of *Haematococcus*. The core of this biotechnology is a two-phase production mode: the first phase involves production under controlled environmental conditions of a high cell density green biomass, followed by a second phase in which this biomass is exposed to extreme environmental conditions, accelerating the encystment process to yield an astaxanthin-rich *Haematococcus* mass.

## 16

### **CRYO-SCANNING ELECTRON MICROSCOPE STUDIES OF THE FUCOID RECEPTACLE**

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Fucoid receptacles were studied with cryo-scanning electron microscopy and X-ray microanalysis as part of studies of the mechanism of gamete release. Receptacles were cryo-fixed in the intertidal zone (*Fucus vesiculosus*, Schoodic Point, Maine) with liquid nitrogen-cooled copper pliers or in laboratory cultures (*Pelvetia compressa*, from California) with liquid nitrogen-cooled ethane or copper pliers. The specimens were held in liquid nitrogen until planed to a smooth transverse face in a cryo-microtome, then examined at -170 °C. The extracellular matrix was sulfur-rich inside the receptacle and chloride-rich between filaments in the medulla. Most cells other than epidermal cells and mature oogonia contained high concentrations of K<sup>+</sup> in the potentiated state (prior to gamete release). Oogonia moving through the extracellular matrix between hair cell filaments during gamete release were compressed. From December-March on the Maine shore, fucoid receptacles are subject to frequent freezing at low tide. Receptacles of *F. vesiculosus* cryofixed on the shore in January (1998) had extensive ice crystal damage. The epidermal, cortical, and receptacle tissues were similar to those of unfrozen receptacles; however, the medullary filaments and extracellular matrix were disrupted and displaced. These findings suggest 1) that fucoidan is confined primarily to the extracellular matrix of the receptacle, and 2) demonstrate why gamete release fails in winter despite the presence of mature gametes.

## 17

### **CONSISTENCY, ACCURACY AND ACCESSIBILITY: PROMOTING COOPERATION AMONG GOVERNMENTAL AGENCIES, MUSEUMS AND TAXONOMISTS**

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Collectively, environmental assessments performed by governmental agencies represent sizable investments for sampling, processing, analyzing and preparing publications as well as a rich source of specimens for taxonomic and biogeographic research. The availability of published collecting and processing methodologies has improved consistency in these areas, but application of nationally consistent and accurate taxonomy for algae in North America has not been achieved. Although efforts such as the Integrated Taxonomic Information System or the National Science Foundation's

Partnerships for Enhancing Expertise in Taxonomy (PEET) Program represent substantial progress, the freshwater algae remain one of the least well-known of the aquatic assemblages taxonomically and biogeographically from a North American perspective. This is of serious concern since the lack of taxonomic resolution and consistency currently limits the uses of algae, which are potentially powerful environmental indicators. Accessibility is linked to accuracy since taxonomic revisions and monographs are more likely to be complete if specialists have access to greater geographic ranges of specimens. Accessibility can be enhanced if governmental agencies routinely deposit material in museums or other depositories and interact with the taxonomic community. Such vouchersing is recycling in its purest form, achieving triple-duty from collections and specimens obtained as part of government-sponsored projects. Not only are (1) the original agency's goals met through its publications, but deposition of specimens and sample remnants provides a (2) "snapshot" of the environment that establishes conditions at a specific time and place and (3) ensures accessibility for taxonomists for revisionary and biogeographic research.

## 18

### **PHYLOGENETIC IMPLICATIONS OF MULTIPORATE TETRASPORANGIAL CONCEPTACLES IN THE PARASITIC CORALLINE RED ALGA, *CHOREONEMA THURETII*.**

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*Choreonema thuretii* (Bornet) Schmitz was placed in the monotypic subfamily Choreonematoideae based on four traits: 1) the absence of either secondary pit connections or cell fusions, 2) conceptacles consisting of only a single layer of cells, 3) the unusual pairing of uniporate tetrasporangial conceptacles with apical plugs, and 4) the presence of spermatangia on both the floor and roof of male conceptacles. Except for members of the newly established subfamily Austrolithoideae, *C. thuretii* is the only coralline red alga with neither secondary pit connections nor cell fusions. However, the recent demonstration of a parasitic habit in this alga casts doubt on this feature as diagnostic due to the extremely reduced nature of the thallus. A second defining characteristic, a conceptacle consisting of only a single layer of cells, is also suspect due both to the definition of cell layers and to the reduced nature of *C. thuretii*. The third characteristic, concurrence of apical plugs and uniporate tetrasporangial conceptacles is shown herein to

be untrue. Although tetrasporangial (bisporangia not seen) apical plugs are present, this study conclusively demonstrates that tetrasporangial conceptacles are multiporate with deeply sunken pore plates. The fourth defining character, presence of spermatangia on both floor and roof of male conceptacle, is confirmed in this study. This combination of new characteristics and reinterpretation of others suggests that *C. thuretii* is a reduced member of the subfamily Melobesiodeae.

## 19

### **A BASELINE STUDY OF PHYTOPLANKTON IN THE UPPER LAGUNA MADRE, TEXAS**

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The Upper Laguna Madre near Corpus Christi, Texas, is a hypersaline estuarine ecosystem which had been afflicted with an extended brown tide algal bloom (*Aureoumbra lagunensis* Stockwell, DeYoe, Hargraves, et Johnson, gen. et sp. nov.). The bloom began in January 1990, and extended through summer 1997. Water column samples were collected at four sites from November 1994 to October 1995. Brown tide population densities were compared with water quality parameters including ammonia, nitrates, orthophosphates, pH, salinity, dissolved oxygen, water temperature, and to other plankton populations including diatoms, dinoflagellates, and zooplankton. Results indicated an inverse relationship between brown tide and diatom population densities. Diatoms were the dominant phytoplankton species and varied at each site. *Chaetoceros* dominated at site 1, *Navicula* at Sites 2 and 3 and *Nitzschia* at Site 4. A total of 51 phytoplankton species and 16 zooplankton species were recorded. The highest diversity occurred at Site 1, the site with the lowest brown tide population. A checklist of organisms, location, dominance, and abundance will be presented.

## 20

### **PORPHYRA SPECIES IN NEW ZEALAND: 18S rDNA SEQUENCING CONFIRMS DIVERSITY**

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New Zealand is an archipelago with an extensive coastline and a large latitudinal range (29° to 54° S). The long geographic isolation of the New Zealand landmass is reflected in a high level of endemism of

both land and coastal marine organisms. The New Zealand coast harbours many species of *Porphyra* (Bangiales, Rhodophyta), both epiphytic (5 species) and epilithic (ca. 12 species). The majority of the epilithic species are as yet undescribed, but systematic studies aimed at delineating and describing these taxa are in progress. Identification of putative species is based on a variety of criteria, including morphology and life history. In the present study we have used sequencing of the 18S rDNA locus to confirm that entities separated on the grounds of life history and morphology are genetically distinct. Our results confirm the presence of a number of species, some of which are presently unnamed, with very divergent 18S rDNA sequences, suggesting long reproductive isolation.

## 21

### CONTRIBUTION OF ALGAL CARBON TO STREAM FOOD WEBS

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Forested streams are generally considered to be heterotrophic, particularly reliant on riparian inputs of carbon and nutrients. We have been studying the importance of riparian vegetation on forest stream ecosystems in North Queensland, Southeast Queensland and Southwest Australia using stable isotope techniques. Rates of respiration and primary production in the streams, measured *in situ* using perspex metabolism chambers, are some of the lowest recorded for forest streams in the world. Despite this, stable isotope analysis of primary sources and consumers suggests that benthic algae play an important role in these stream ecosystems. In this paper we examine several of the factors that may influence the relative importance of benthic algae to stream food webs in different regions of Australia.

## 22

### TWO CO-OCCURRING SPECIES FROM THE "TOXIC PFIESTERIA COMPLEX: " COMPARATIVE N/P STIMULATION AND ICHTHYOTOXICITY

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We compared the response to N and P enrichments, and toxicity toward fish of the toxic dinoflagellate, *Pfiesteria piscicida* and a second *Pfiesteria*-like species (not yet formally named) that were isolated (unispecies but not axenic) from two known fish kill sites in the Neuse Estuary, NC. Both dinoflagellates were acclimated in separate cultures for two months under similar conditions in which they were (a) grown with fish or (b) grown with a cryptomonad as algal prey. Nontoxic zoospore production (i.e., in the absence of fish) was then compared for each species in separate trials across gradients of nitrate, ammonium, inorganic phosphate, and organic phosphate enrichment in short-term batch cultures, with or without an N/P-starved cryptomonad as available algal prey (sufficient prey added to achieve an initial concentration of 5,000 or 50,000 cells mL<sup>-1</sup>). The two species differed significantly in response to both fish and nutrients/algal prey, in timing and overall cell production. *Pfiesteria piscicida* responded more slowly and attained significantly lower cell densities when given algae, especially following a diet of live fish. Highest growth of both dinoflagellates occurred with nitrate stimulation and tracked growth of the cryptomonad prey. Our isolate of *P. piscicida* was significantly more toxic to fish than the *Pfiesteria*-like species, with 100-fold higher cell density or much longer periods required for lethality from the *Pfiesteria*-like species. Thus far the data indicate that the second known *Pfiesteria*-like species isolated from natural habitat is only weakly toxic to fish, relative to *P. piscicida*.

## 23

### ONTOGENETIC DYNAMIC OF THE ABSOLUTE CONFIGURATION OF THE FLAGELLAR APPARATUS OF MALE GAMETES OF *CHARA CONTRARIA* VAR. *NITELLOIDES* (CHARALES, CHAROPHYTA)

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The ontogenetic dynamic of the absolute configuration of the flagellar apparatus of male gametes of *Chara contraria* var. *nitelloides* (Charales, Charophyta) was studied. During the different stages of the male gametogenesis the following traits were carefully analyzed: basal bodies angles and lengths, basal bodies intermediate zone, microtubular roots angles and

lengths, "Vierergruppe", distal and proximal fibers, proximal sheaths, flagellar length, flagellar transversal profiles and flagellar tips, etc. Conventional transmission electron microscope and serial sectioning techniques were used. Three principal stages have been found: i) parallel basal bodies connected by a proximal non-striated fiber, ii) basal bodies connected by a distal striated fiber and iii) basal bodies without connection fibers. The fact that during the very inception of the development of the basal apparatus the basal bodies are parallel to each other and perpendicular to the nuclear envelope is discussed from an evolutionary point of view.

## 24

### **HETEROSIGMA CARTERAE: A MODEL SYSTEM FOR HARMFUL ALGAL BLOOM STUDIES**

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Three areas of research have been targeted using the Raphidophyte, *Heterosigma carterae*, as a model system: 1) Genetic fingerprinting: Sequence analysis demonstrates that bio-geographically disparate populations of the alga encompass a single species. Taxon-specific DNA hybridization probes have been developed to distinguish this pleomorphic cell from other biflagellate, toxic algae (e.g. *Fibrocapsa japonica*, *Chattonella japonica*, *Olisthodiscus luteus* and *Cryptomonas* sp.); 2) Life history profiles: Environmental triggers impact both cell survival and toxin biogenesis. Vegetative cells have been induced to form non-motile 8-10 mm "cysts" with 60-70% efficiency by manipulating light, temperature and nutrient loads. "Cysts" kept in the dark for 19-35 days can excyst when exposed to favorable environmental conditions. Unlike these putative "cysts", a large, long-lived 18mm "spore" phase, is induced solely by alterations in irradiance. Although the toxicity of these resting phases is presently unstudied, vegetative cells produce a family of allelopathic compounds that kill eco-cohorts at every trophic level. *Heterosigma* is capable of sequestering a hemolytic toxin of unknown molecular identity. 3) Cell function: *Heterosigma* is an obligate photoautotroph. The relationship between light availability and the capacity of this alga to enter into an active reproductive phase or to transform into a "cyst" or into a "spore" is well established. We hypothesize that light may serve as a cue which drives *Heterosigma* cell function. Using *Heterosigma* as a model experimental system, we have begun to document

unique aspects of chromophyte cell function i.e. to probe the molecular circuits responsible for light regulation of gene expression and to unravel the biochemical pathways that affect metabolic change.

## 25

### **AN ANALYSIS AND INTERPRETATION OF WATER CHEMISTRY, SUBSTRATE, AND DIATOM COMMUNITIES IN MOUNTAIN LAKE, VIRGINIA**

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Mountain Lake is the only natural lake in the unglaciated southern Appalachian Highlands. It is an oligotrophic to meso-oligotrophic freshwater system traditionally exhibiting an oligotrophic algal flora, including sparse diatoms of relatively low diversity in plankton collections, and *Nitella megacarpa* and *Elodea* as native macrophytes. In recent years, the lake has exhibited early signs of eutrophication, as well as the increasing presence of the non-native macrophyte *Ceratophyllum demersum*. Our present study of lake water chemistry and nutrients shows the system to be largely controlled by phosphorus, and to a lesser extent by nitrate concentrations, from specific individual input streams and rainwater. Diatoms from modern sediment show different and more diverse forms than those from either plankton or diatometer collections from the lake. Several previously unreported forms are noted. The sediment diatoms comprise several individual thanatocommunities related to water depth, substrate, and nutrient conditions associated with individual stream sources. In addition, native macrophytes show more diverse epiphytic community associations than does *Ceratophyllum*, which is associated here with a low diversity, near monoculture of *Cocconeis placentula*. These results are being applied to lake sediment core analysis to determine past depth, environmental and nutrient conditions within the lake.

## 26

### **STRAIN IMPROVEMENT OF SEAWEEDS FOR AQUACULTURE THRU BIOTECHNOLOGY: CURRENT STATUS AND FUTURE PROSPECTS**

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The seaweed industry of developed countries like the United States has always been based upon the harvesting of wild native species or cultivated foreign species. This needs to change if the industry is ever going to expand in the US and other developed countries. One way this can be done is by developing aquaculture systems of genetically modified/improved seaweed strains that produce novel, higher-valued products that can not be obtained from native or foreign wild plants. Until recently there was little hope that this can be done. However, as a result of recent advancements in protoplast fusion and analogous techniques, we have developed practical methods for the genetic modification and improvement of virtually any commercially-valuable red macroalga. To date, we have produced new strains of the two major carrageenophytes under cultivation, *Eucheuma denticulatum* and *Kappaphycus alvarezii*, which demonstrate significantly faster growth rates (up to 14% per day) in extensive field trials. We have also produced a hybrid/polyploid between the Japanese cultured species *Porphyra yezoensis* (nori) and a North Atlantic species, *P. umbilicalis*, which exhibits an increase in chromosome number and faster growth rate. Future plans are to produce transgenic nori that can serve as an "edible vaccine" when eaten.

27

**PHYLOGENETIC RELATIONSHIPS AMONG SELECTED CERAMIALES (RHODOPHYTA) BASED ON NUCLEAR SMALL-SUBUNIT rDNA SEQUENCES: FOCUS ON THE DASYACEAE, DELESSERIACEAE AND RHODOMELACEAE**

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Previous molecular investigations of nuclear small-subunit (SSU) rDNA sequences have indicated that the Ceramiaceae is paraphyletic in excluding a monophyletic clade including the Dasyaceae, Delesseriaceae and Rhodomelaceae. Furthermore, it is generally accepted that these latter three families are themselves monophyletic. However, only one species for each of the Dasyaceae, Delesseriaceae and Rhodomelaceae have been included in previous SSU molecular investigations. The aim of the current investigation is to test the general acceptance that these three families are monophyletic by generating a more robust SSU phylogeny for the

Ceramiaceae. Preliminary data, based on an SSU alignment for 36 ceramiacean algae including 12 ceramiacean, 12 dasyacean, 6 delesseriacean, and 6 rhodomelacean species, remain consistent with the notion that the Dasyaceae, Delesseriaceae and Rhodomelaceae have evolved from a common ancestor within the Ceramiaceae. However, our data indicate that the Rhodomelaceae alone is monophyletic with the Dasyaceae polyphyletic and the Delesseriaceae paraphyletic. The implications for taxonomy in the Ceramiaceae will be discussed.

28

**INVESTIGATIONS OF PHYCOCOLLOID BIOCHEMISTRY AS A CHEMOTAXONOMIC INDICATOR OF PHYLOGENY IN THE GIGARTINALES**

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The taxonomic significance of the polysaccharide structures of the algal cell walls has been underlined several times over the past few decades but never pursued in a systematic manner because, as McCandless put it in 1978 "... division on the basis of constituent polysaccharides is either a taxonomist's nightmare, if one looks at the changes in classification which would have to result, or a biochemist's nightmare, if one looks at the existing classification and considers the analyses still to be done"! Many changes in red algal systematics and the biochemical analyses of phycocolloids have developed since her statement. We are testing whether the method developed by Chopin and Whalen for carrageenan identification by FT-IR diffuse reflectance spectroscopy can be extended to the study of phycocolloids for diverse species of many red algal orders. Its rapid screening capability (directly on dried, ground algae) and minimal requirement of material (only a few milligrams) should reduce the "nightmarish" aspect of this long-term investigation. Results of FT-IR diffuse reflectance spectroscopy will be considered in light of recent molecular phylogenies to determine if key phycocolloid attributes can be identified that correspond with familial and ordinal level groupings. Biochemical alternation of generations is well documented in species of the Gigartinales and Phyllophoraceae, with either isomorphic or heteromorphic life histories, studied to date. A secondary aim of this study is to determine if this pattern holds as more species of these two families are

investigated, and to ascertain if a similar situation occurs for species of other families and orders.

## 29

### NOVEL INSIGHTS INTO THE PHYLOGENETIC AFFINITIES OF *KATABLEPHARIS OVALIS* & *KATABLEPHARIS PHOENOKISTRON* (KATABLEPHARIDACEAE) SKUJA.

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The colorless flagellate *Katablepharis* Skuja is comprised of six species based on light microscopic studies. Two of the species, *K. ovalis* and *K. phoenokistron*, are compared at the light microscopic and ultrastructural level. For *K. phoenokistron*, this represents the first ultrastructural study. Although the two species share most cellular characteristics, including a feeding apparatus, alveolar-like sacs associated with subpellicular microtubules, ejectisomes, and cell cover, they differ in some respects. First, observing the cytopharyngeal rings within the feeding apparatus reveals that *K. ovalis* possesses two whereas *K. phoenokistron* possesses nine to ten rings. Secondly, *K. ovalis* ejectisomes are comprised of a single coil that when forcibly discharged produces a single coiled tube structure. In contrast, *K. phoenokistron* appears to harbor, in addition to the type found in *K. ovalis*, ejectisomes with a large coil attached to a small coil or subsidiary scroll. These bipartite ejectisomes, when discharged, form a long tube with a small attached tube deflecting out at a circa 45° angle. The question of primitive or advanced regarding the two species is addressed by comparing them to other protist groups that also share these structural features. In addition to intrgeneric comparisons, larger scale phylogenetic relationships for *Katablepharis* are explored. Based on ultrastructural studies, an argument is presented for a *Katablepharis*/cryptomonad relationship. Also, a *Katablepharis*/apicomplexan relationship is suggested, based on the feeding apparatus and the alveolar-like structures. The proposals are based upon evidences utilizing comparative ultrastructure, monoclonal antibody studies, and phylogenetic studies utilizing the 18S ssrRNA gene sequence for *Katablepharis ovalis*.

## 30

### IS DNA SEQUENCE INFORMATION USEFUL AT THE SUBSPECIES LEVEL?

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The limits of the value of sequence data for reconstruction of phylogenetic history are still being explored. The utility of nuclear rDNA internal transcribed spacer region sequences (ITS-1 and ITS-2) has been limited by uncertainties of alignment. With the discovery of the secondary structure of the transcripts of these regions, more valid alignment is possible. To examine the taxonomic lower limits of this information, we have sequenced 18 isolates of *Gonium pectorale*, a morphologically uniform species of worldwide distribution. After alignment guided by secondary structure, we assessed their relationships by parsimony and by nearest neighbor methods. Both give the same phylogram. We also assessed their mating relationships, both for zygote production and for germination and survival of products from zygotes. All isolates fall, by mating behavior, into one of two complementary mating types that appear to characterize the species. No member of the species has been found to mate with any other *Gonium* species, even the most closely related by sequence, *G. octonarium*. Within *G. pectorale*, there are two major clades by sequence analysis. Mating and zygote formation can occur between representatives of these two clades, but no zygote products result. The mating data and the degree of F1 survival parallel precisely the relationships suggested by the sequence data phylogram. Thus, ITS sequence data seem suitable for subspecies analyses as well as species and generic studies.

## 31

### 18S PHYLOGENY OF CRYPTOMONADS

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Cryptomonads are a distinct group of unicellular algae that have traditionally been classified according to their morphology and colour. They are something of a curiosity because they have obtained their plastid by engulfing and permanently maintaining a photosynthetic eukaryote (the endosymbiont). We have sequenced 12 nuclear 18S ribosomal RNA genes from species that represent a broad range of cryptomonad morphological and pigment diversity. By analyzing these new 18S rRNA sequences with 11 cryptomonad sequences completed by previous investigators, we have been able to identify a number of well supported assemblages within cryptomonads. Blue/green cryptomonads that contain phycocyanin pigment form a clade distinct from other species that contain phycoerythrin pigment. Species with a nucleomorph (the endosymbiont nucleus)

embedded in the pyrenoid form a well supported clade. Species containing type II phycoerythrin group with the colourless cryptomonad *Chilomonas* suggesting that *Chilomonas* evolved by pigment loss from a species that contained type II phycoerythrin. There are a number of species that do not group together strongly but are similar in that they have a single endosymbiont with a free nucleomorph. This configuration appears to be an ancestral state that still persists in some cryptomonad lineages. We have also obtained 18S rRNA sequence for a second species of the plastid lacking heterotroph, *Goniomonas*, and our analyses confirm that *Goniomonas* spp are related to the cryptomonad host cell, but diverged before an endosymbiont with a plastid was acquired.

### 32

#### **RE-EVALUATING THE "OVERFLOW" HYPOTHESIS: WHAT IS THE LINK BETWEEN PHOTOPIGMENT & 2-METHYLISOBORNEOL SYNTHESIS IN CYANOBACTERIA?**

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Many cyanobacteria produce the terpenoid metabolite 2-methylisoborneol (1-R-exo-1,2,7,7-tetramethyl-bicyclo-[2,2,1]-heptan-2-ol; MIB) that exhibits a characteristic 'muddy' taste and odor. Although non-toxic, MIB is considered a significant problem in the aquaculture industry and municipal water supplies because fish and/or potable water containing MIB are undesirable and often misconstrued as unsafe. Previous research has indicated that the cell content of MIB accumulates in response to inhibition of the isoprenoid biochemical pathway; thereby suggesting that MIB biosynthesis provides a unique mechanism to dissipate excess carbon in the terpenoid/pigment pathway via an 'overflow' mechanism. In a series of culture experiments, chemically-induced inhibition of the terpene pathway 'below' and 'above' the branch points of terpenoid and photopigment synthesis, respectively, in conjunction with varying amounts of photosynthetic active radiation resulted in dramatic alterations of photopigment content/composition within *Oscillatoria chalybea* and *Pseudanabaena articulata*. However, cellular and total accumulations of MIB were not altered by these physiological/environmental perturbations, even when these perturbations were imposed at nearly lethal concentrations/levels. In addition, cyanobacterial cultures maintained in the laboratory often are observed to reduce or cease MIB biosynthesis; thereby indicating that MIB production is not a requirement for active

growth. As such, the lack of coupling between MIB and algal growth and/or photopigment alteration suggests that MIB production may not be a direct means to dissipate "excess" carbon as has been previously postulated.

### 33

#### **ION REGULATION OF MEMBRANE TRAFFICKING AND MUCILAGE SECRETION IN THE DESMID, *CLOSTERIUM***

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The desmid, *Closterium* sp., possesses a complex mucilage-secretory pathway that includes an elaborate endomembrane system (ES) and an active, cytoplasmic streaming network (CSN). Central to the ES is a network of over 100 Golgi bodies, each consisting of a well-defined stack of 12-15 cisternae and various secretory vesicles. An extensive but incompletely described network of F-actin cables is a key component of the CSN. In this study, an experimental and immunocytochemical examination of the ES and CSN was undertaken. Treatment with 0.01 $\mu$ M of the sodium ionophores, monensin and SQL, resulted in Golgi alteration that included typical cisternal curling and trans face swelling. Immunogold labeling using an anti-mucilage antibody showed that mucilage processing through the Golgi Apparatus was unaffected for the first hour of treatment. Monensin and SQL also caused localized formation of wall inclusions in the polar zones. These structures are presumably caused by alterations of wall-vesicle fusion sites at specific zones of the plasmamembrane. Likewise, actin cables coalesced together to form brush-like aggregates and cytoplasmic streaming subsequently ceased. Treatment with calcium channel blockers like verapamil, resulted in CSN cessation and cisternal fusion of the trans face locus of the Golgi Apparatus. Treatment with calmodulin antagonists such as W7, TMB-8 and TFP caused a distinct formation and clustering of coated vesicles at the trans Golgi region. Mucilage secretory vesicles were produced for the first 15 min. of treatment. For longer periods of treatment, secretory vesicle production was halted. A model to explain the multiple roles of specific ions in the secretory network will be presented.

### 34

#### **GENETIC INVESTIGATIONS OF TWO DIVERGENT MORPHOLOGIES OF *CHONDRUS CRISPUS* (RHODOPHYTA) USING AMPLIFIED FRAGMENT LENGTH POLYMORPHISM (AFLP)**

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Two morphologies of *Chondrus crispus* Stackhouse (Rhodophyta) have been described in the Maritime provinces of Canada: N (narrow) and B (broad). Hybridization attempts were unsuccessful, possibly indicative of genetic distinctiveness of these two forms, however, environmental forces have also been implicated as causing the morphological divergent of the N and B varieties. No genetic analyses to date have been conclusive at resolving population-level genetic differences between these two phenotypically divergent stands of *C. crispus*. Amplified Fragment Length Polymorphism (AFLP) is assessed as a method for resolving genetic structure between the N and B morphologies of *C. crispus* found in the Maritime provinces of Canada.

### 35

#### **BACTERIAL INFLUENCES ON HAB POPULATION DYNAMICS**

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It is well-documented that the characteristics of a bacterial assemblage change in both a quantitative and qualitative sense over the course of an algal bloom. It has also been suggested that the microbial community may play either a beneficial or detrimental role in regulating algal growth, often in a species-specific manner. However, as a potentially important factor regulating the population dynamics of harmful algal blooms (HABs), bacteria have received relatively little attention. Recent emphasis on HAB growth regulation, both from a modeling/prediction perspective, as well as the development of strategies for bloom mitigation and control, has provided a new impetus for studies of bacterial-algal interactions and their influence on HAB dynamics. Our current research is directed at the isolation and characterization of bacteria either lethal to (i.e., algicidal), or adversely affecting the growth of HAB species, with emphasis on the mechanism of algicidal activity and the basis of taxonomic specificity for the target algal species. In addition, we are attempting to discern to what extent interactions between a given HAB species and its associated bacterial assemblage may shape the composition of the latter. An overview of work in this area, as well as results from

our recently initiated work focusing on the Florida red tide dinoflagellate, *Gymnodinium breve*, and its associated bacteria, will be presented.

### 36

#### **ROLE OF FIRE ASH ON NUTRIENTS AND FOODWEB DYNAMICS IN A SOUTHWESTERN USA FOREST STREAM**

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We tested the short and long term responses of fire-ash on a first order stream in the Gila National Forest in New Mexico, USA. Three hundred gallons of fire-ash slurry, from a nearby wild fire, was delivered to Meadow Creek over a 75-minute period. Selected physico-chemical and biotic responses were monitored at upstream (control) and downstream (ash treatment) sites prior to, during, and at 3 h, 22 h, 1 mo, 2 mo, and 9 mo intervals after the ashing event. All major ions and nutrients showed a significant ( $p \leq 0.01$ ) increase in concentration during the ashing event with recovery to control levels within 3 h of the event, except for K, NH<sub>3</sub>, and NO<sub>3</sub>, where recovery took up to 22 h after treatment. Elevated concentrations of PO<sub>4</sub> continued to be detected at 2 mo following the event. Hydrogen-ion concentration increased from 7.7 to 8.9 during the ashing event, but recovered within 22 h after treatment. Dissolved oxygen concentration decreased from 6.3 mg L<sup>-1</sup> to 5.0 mg L<sup>-1</sup>, also recovering to control levels within 22 h after treatment. There was no significant difference between control and treatments for periphyton biomass, and only minor compositional changes in the phytobenthic community. Mixed responses were seen in the invertebrate community, where mayfly nymphs, Hemiptera, and select members of caddis flies appeared to be sensitive to the ashing event. The importance of forest fires in the maintenance of stream structure and function will be discussed.

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#### **KELP RECOVERY FOLLOWING LONG-TERM KELP CANOPY EXCLUSION**

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Experiments in Carmel Bay, CA indicated that kelp canopy exclusion led to increased cover of perennial turf algae and decreased availability of bare space. To examine if these changes affected kelp recovery following the study, recruitment of the kelps

*Macrocystis pyrifera* and *Pterygophora californica* was monitored in the exclusion areas (old) and in newly established canopy clearings. Recruitment of *M. pyrifera* did not differ between the clearing types while *P. californica* recruitment was greater in newly established clearings. Removal of turf algae from experimental plots within the clearings led to similar patterns of recruitment between canopy treatments. Survivorship of kelps that recruited epiphytically onto turf algae was lower than of those that recruited onto bare space. *Macrocystis pyrifera* recruitment was evenly dispersed throughout all of the clearings, while *P. californica* recruitment was evenly dispersed in the new clearings, but concentrated near the edges of the old clearings. Further implications of kelp canopy exclusion will be discussed.

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**EVIDENCE FOR A MICROARCHITECTURE AND ASSOCIATED ORGANIC MATRIX IN THE DIATOM FRUSTULE**

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Diatoms are encased in an intricate and highly ordered frustule that is constructed by precise temporal and spatial patterns of silica deposition. We are investigating the biological processes involved in the formation of the frustule, as these three-dimensional composites make ideal systems for modelling the design and synthesis of novel materials for advanced technology. To date, conventional scanning and transmission electron microscopy (including electron diffraction) data suggest that the frustule is relatively unstructured, composed of amorphous silica and at best differentiated as ribs or a honeycomb system. Here, we present field emission scanning electron microscopy (FESEM) and atomic force microscopy (AFM) data that illustrates the presence of a highly organized microarchitecture within the frustule that has not been described previously. A finely-patterned microarchitecture was observed primarily within the valve and to a lesser degree within the girdle bands; these structures remain embedded within the heavily silicified regions of the frustule. In addition, AFM imaging coupled with <sup>13</sup>C-nuclear magnetic resonance (NMR) analyses of acid-washed frustules indicate the presence of an organic matrix material embedded within heavily silicified structures. Together, these

observations indicate that an organic matrix or template may be directly involved with biomineralization, and that this material remains associated with the microarchitecture (i.e., as a scaffolding) following cell wall development.

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**MICROALGAL XANTHOPHYLL-CYCLE PIGMENT POOLS AND FUNCTIONALITY AS A FUNCTION OF THE SPECTRAL DISTRIBUTION OF STATIC GROWTH LIGHT**

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The effects of high and low, white-light upon xanthophyll-cycle pigments has been well studied. However, aquatic light fields present highly unique spectral distributions/photon flux densities that can change on relatively short time scales. We have begun a series of experiments that will address the effects of static and dynamic growth-light upon xanthophyll-cycle functionality in microalgae. To address the question of static growth-light we observed the effects of three spectrally distinct growth light regimes upon the pool-size and functionality of the xanthophyll-cycle pigments of the Chrysophyte, *Ochromonas danica*. A diffuse white and two diffuse, colored (blue-green and red) filters were used in conjunction with neutral density screening to provide at least three photon flux densities under each of the three spectral distributions. Pigment epoxidation/de-epoxidation kinetics (HPLC), PSII fluorescence (PAM), oxygen evolution and in vivo absorbance characteristics were monitored during both an increasing and decreasing photon flux density in a modified photosynthesis/irradiance (P/E) cuvette. Maximum xanthophyll-cycle induction/relaxation kinetics also were determined. Intercomparison of growth light regimes was based upon photosynthetically absorbed radiation (PHAR), as opposed to photosynthetically available radiation (PAR).

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**DIVERSITY OF DINOFLAGELLATES AT PELICAN CAYS, BELIZE**

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This study examined the diversity of toxic and non-toxic marine dinoflagellates in six relatively pristine,

biologically diverse, semienclosed, oceanic mangrove islands situated within the Pelican Cays Archipelago, Belize. The atoll-like Pelican Cays exhibit enclosed, different sized lagoons, associations of mangrove corals and seagrass beds, and relatively stable oligotrophic conditions. These islands are circular depressions, that are separated from the outside ocean by steep coral ridges, that restrict the exchange of water. Dinoflagellate assemblages were examined in 46 plankton net tow samples collected at Cat Cay, Douglas Cay, Elbow Cay, Fisherman Cay, Lagoon Cay, and Manatte Cay in May, 1994. The associations of dinoflagellates are documented in SEM pictures. I have identified 58 taxa: *Amphidinium* (3), *Ceratium* (8), *Cochlidinium* (1), *Cryptodinium* (1), *Dinophysis* (2), *Diplopelta* (1), *Diplopsalopsis* (1), *Gambierdiscus* (2), *Gonyaulax* (5), *Gymnodinium* (5), *Ostreopsis* (4), *Phaeopolykrikos* (1), *Podolampas* (2), *Prorocentrum* (5), *Protoperdinium* (10), *Pyrodinium* (1), *Pyrophacus* (3), and *Scrippsiella* (3). The diversity of dinoflagellates is compared between six Pelican Cays oligotrophic habitats with four mesotrophic mangrove habitats, part of the Atlantic barrier reef-mangrove ecosystem.

#### 41

### PHYLOGENETIC ANALYSIS OF COCCOID PRASINOPHYTES (CHLOROPHYTA) USING 18S rDNA SEQUENCE DATA.

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18S rDNA sequences have been obtained for 8 isolates of marine coccoid green algae. Phylogenetic analyses using maximum parsimony, maximum likelihood, and neighbor-joining methods indicate that prasinococoids are part of a lineage that includes other prasinococoid algae, such as *Mantoniella*, *Pseudoscourfieldia*, and *Micromonas*. Our results suggest that prasinococoid green algae form a monophyletic group basal within the green algae. Our results are also in agreement with previous studies that have indicated that the Prasinophyceae as a whole are not monophyletic.

#### 42

### SIZE-DEPENDENCE OF METABOLIC PROCESSES IN MARINE DIATOMS (BACILLARIOPHYCEAE) UNDER LIGHT-LIMITING CONDITIONS

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The light-limited size-dependence of photo-synthesis, respiration, exudation, growth, quantum yield, and light absorption was quantified for eight species of centric marine diatoms: *Chaetoceros calcitrans* (Paulsen), *Thalassiosira pseudonana*, *Chaetoceros* sp., *Thalassiosira weissflogii* ((Grunow) Fryxell et Hasle), *Hyalodiscus* sp., *Planktoniella* sp., *Coscinodiscus* sp. (Ehr. (1839)), and *Coscinodiscus* sp. The size scaling exponent associated with the photosynthetic rate is significantly smaller than the commonly reported exponent of  $-1/4$ . In contrast, carbon-specific respiratory loss and the specific growth rate have size-scaling exponents close to  $-1/4$ . Exudation does not have a statistically significant relationship with cell size. In contrast to growth and photosynthetic processes, growth and photosynthetic efficiencies are independent of cell size. The size-dependence of light absorptive properties provides a mechanism to explain the anomalous size scaling exponents associated with anabolic rates. The robust statistically significant relationships between cell size and photosynthesis, respiration, and growth was confirmed, but the magnitude of the size scaling exponent associated with light-limited anabolic processes indicate that the allometric power law with an exponent of  $3/4$  does not apply to photosynthetic organisms under all environmental conditions.

#### 43

### TAXONOMIC STATUS OF THE ANTARCTIC RED ALGA *KALLYMENIA ANTARCTICA* HARIOT

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One of the common red algae encountered along the Antarctic Peninsula and around the South Shetland Islands is *Kallymenia antarctica* Hariot 1907. Detailed morphological studies based on recent collections from King George Island in the South Shetland Islands were undertaken to characterize the vegetative and reproductive morphology of this species and to assess its taxonomic placement. The sequence of the chloroplast-encoded *rbcl* gene was compared with sequences from other red algae worldwide. Morphological and molecular data indicate that the species is misplaced in *Kallymenia*, and point to the genus *Sarcodia* (Sarcodiaceae, Gigartinales) as an appropriate placement.

## 44

**GENETIC DIVERSITY OF GREEN LICHEN PHOTOBIONTS AND EVOLUTION OF GROUP I INTRONS IN LICHEN SYMBIOSIS**Friedl, T.<sup>1</sup> & Bhattacharya, D.<sup>2</sup><sup>1</sup>*University of Kaiserslautern, Kaiserslautern Germany;*<sup>2</sup>*Department of Biological Sciences, University of Iowa, Iowa City, IA 52242 USA*

Members of the most abundant lichen algae, *Trebouxia* spp., as well as non-lichenized taxa of the green algal class Trebouxiophyceae are particularly rich in SSU rDNA group I introns. Phylogenies inferred from intron sequences were consistent with rDNA phylogenies of free-living trebouxiophytes demonstrating a stable vertical inheritance of the introns in these algae. To investigate the relationships between the evolutionary histories of group I introns and the genomes in which they reside, ITS rDNA sequences were determined for various strains and species of *Trebouxia*. Secondary structure models were used to refine the alignment of ITS and intron sequences. These analyses suggest that introns which were vertically inherited among the free-living members of the Trebouxiophyceae are often laterally transferred when these algae become lichenized. Lichenization may facilitate lateral transfer among the bionts due to their intimate cell-to-cell contact within the lichen thallus. Phylogenetic and secondary structure analyses of the ITS sequences revealed a clear division of species previously attributed to the genus *Trebouxia* into two genera, *Trebouxia* and *Asterochloris*. For *Trebouxia* spp., a distinction into four lineages of closely related species is resolved. These evolutionary relationships are in agreement with morphology and ultrastructure. Also, a considerable intraspecific variation was found and may be correlated with geographic isolation and/or with different types of mycobionts with which the photobionts are associated.

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**CAROTENOIDS FROM DUNALIELLA: A TRUE ALGAE BUSINESS**

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Since the 1950's there have been numerous efforts to turn the promise of algal productivity and biochemistry into real businesses. Not surprisingly, this has resulted in more failures than successes. Carotenoid pigments

from the microalga, *Dunaliella salina*, can be counted as one of the successes. In recent years the industry has matured and consolidated so that there is considerable cooperation between producers and more information about their operational details. This has been crucial in light of the research results on the relationship of carotenoids (especially beta-carotene) to human nutrition and disease over that time. The presentation will compare and contrast three of the largest producers: Nutrilite/Amway in southern California, Betatene in Australia, and Nature Beta Technologies in Israel. Comparisons will be made in the areas of algal strains, medium, growth systems, harvesting, extraction, product formulation and marketing. All are different, yet all are successful. What lessons can be learned to help move other algal product ideas from the laboratory to industry?

## 46

**DETECTION OF THE TOXIC PFIESTERIA COMPLEX: A TECHNIQUE COMPARISON**

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Two methods are currently in use for culturing the ichthyotoxic *Pfiesteria* species "complex": toxic stages and species are detected using bioassays with live fish in biohazard III facilities or, alternatively, nontoxic stages (as well as other mixotrophic species) are cultured using algal prey. We compared zoospore production for *Pfiesteria piscicida* and a second toxic *Pfiesteria*-like species (thus far, more weakly toxic and not yet formally named) using these two culture methods. Eight field samples from Chesapeake Bay and the Albemarle-Pamlico were sub-aliquoted (n=3), and subsamples were cultured with fish or with cryptomonad prey over a 8-week time series. At least 100 cells prepared with suture-swelling procedures were rotated to determine plate arrangement for morphospecies identification (ca. 2,500 cells screened over 2.5 days per replicate). In 7 of 8 comparisons, the fish bioassay yielded only *P. piscicida*; in 1 case, a mix of *P. piscicida* (60%), the 2nd toxic *Pfiesteria*-like species (10%), and *Gyrodinium galetheanum* (20%) occurred. By contrast, algal bioassays consistently yielded species mixes that were dominated by various nontoxic species or by the second potentially toxic *Pfiesteria*-like species, with *P. piscicida* less common or not detected. The data indicate that (i) the algal assay is not reliable for detecting *P. piscicida*; and (ii) under the culture conditions used, *P. piscicida* outcompetes other algae in the presence of live fish, whereas the 2nd potentially

toxic *Pfiesteria*-like species and other mixotrophic dinoflagellates outcompete *P. piscicida* for algal prey.

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#### TRACES OF POSSIBLE TRANSPOSON ORIGINS OF RED ALGAL PLASMIDS.

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Many red algae harbor circular DNA plasmids in their cells. Recent *in situ* PCR localization studies have shown that the plasmids of *Gracilaria* and *Gracilariopsis* reside in host nuclei. They are of interest because of their potential use as shuttle vectors for genetically altering red algal genomes. Red algal plasmids occur at very high copy number (.,100 copies per cell), they are highly conserved at the species level and generally two or more plasmids co-reside in host cells. This is the case in Chilean *Gracilaria chilensis* which harbors both Gch3937 (GC1) and Gch3827 (GC2). However, *G. chilensis* from New Zealand harbors only a single plasmid, Gch7220. Analysis of the sequence of Gch7220 shows that this plasmid is a chimera of the two plasmids present in the Chilean population. The entire Gch3937 plasmid has been inserted into a conserved ORF of Gch3827 to form the larger Gch7220 plasmid. The formation of the chimeric plasmid appears to have involved a homologous recombination event between a short 70 bp region of DNA. Recombination results in a crossover between the two plasmids at the insertion point. As a result, Gch3827 is positioned between the two homologous regions and the sequence of Gch3827, immediately adjacent to these homologous regions are an exact repeat of CTTT and this repeat flanks a short 5 bp inverted repeat sequence: CTTAG and CTAAG. This feature - the presence of flanking short exact copies bordering short inverted repeat sequences - is a hallmark of transposable elements following insertion events.

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#### GENETIC POLYMORPHISM IN THE ITS REGION OF 15 STRAINS OF *DUNALIELLA*

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The ITS region of 15 strains of *Dunaliella* (13 belonging to the section *Dunaliella* and 2 to the section

*Tertiolectae*) was studied by RFLP. The DNA was obtained from unialgal cultures and after PCR amplification, the ITS was, in some cases, further purified using a DEAE-cellulose NA45 membrane. The ITS fragment from each strain was single and double digested with the following endonucleases: Ava II, Ban II, Bsr I, Dde I, EcoRV, Hae III, Hha I, Hind III, Hinf I, Msp I, Pvu II and Sty I. A cluster analysis was assessed based on the presence or absence of bands generated by RFLP, using the NTSYS software package. A genetic map of the ITS was also performed for each strain. Even though the length of the fragment was the same for most of the strains (ca. 680 bp), the restriction patterns obtained showed variable fragment divergencies within and among species. Except for the strain CCMP 1303 of *D. salina* which exhibited an ITS region ca. 180 bp shorter than any of the taxa studied and a restriction pattern also completely different, the ITS region appears to be an appropriate genetic marker to discriminate strains within *D. salina*. An unexpected similarity of the restriction pattern was found between 2 strains of *D. parva* and one strain of *D. tertiolecta*, even more close than among the strains of *D. salina*. The genetic polymorphism found among taxa will be discussed in term of inherent genetic variation and erroneous identification of some strains coming from culture collections.

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#### BIOGEOGRAPHY OF THE CYANIDIACEAE (RHODOPHYTA) BASED ON 18S RIBOSOMAL RNA GENE SEQUENCE DATA

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Volcanic areas with pH-values ranging from 0.5 to 4 and temperatures up to 56 °C are inhabited by three unicellular red algae: *Cyanidioschyzon merolae*, *Cyanidium caldarium*, and *Galdieria sulphuraria*. These highly acidic habitats are scattered all over the world and are usually very small. Because all three red algae are acido- and thermophilic and will not tolerate desiccation, a distribution by wind or water seems very unlikely. Therefore, the various populations might have been isolated for very long times. We investigated the biogeography of 10 isolates of these red algae by sequence comparison of the 18S RNA. A gene tree places *C. merolae* and *C. caldarium* in a sister clade to the Bangiales and to higher red algae. At the base of this branch, the *Galdieria*-isolates form a separate branch which further divides into four groups

representing different geographical areas. This indicates that at least the genus *Galdieria* developed several geographical races with significantly altered 18S sequences. Therefore, a long separate evolution of these isolates seems likely.

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**QUALITATIVE AND QUANTITATIVE STUDY OF TROPICAL BENTHIC MACROALGAL POPULATIONS FROM COMMUNITIES UNDER ANTHROPOGENIC DISTURBANCE.**

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This study was carried out on the Praia Rasa shore, Buzios City (Cape Frio, Rio de Janeiro State, Brazil), which has experienced a conspicuous increase of human activity during the last decade. The aim was to evaluate the anthropogenic impact on the region's macroalgal communities by comparing current data with (1) previously published results obtained before the establishment of human presence in the area, and (2) along a pollution gradient. Techniques were conducted in the Rhodophytan zone in three established sample sites where a total of 97 macroalgal species were found. Qualitative and quantitative variation were detected in local communities, especially at sites closer to the anthropogenic disturbance source. There, total algal cover values and species composition changes decreased, pollution-sensitive species such as *Caulerpa racemosa* disappeared whereas others, such as *Ulva* spp. increased in abundance. Total number of species and reproductive index did not seem to have undergone significant changes over the years. Cluster and factor analysis (FA) revealed that of the three sample sites, the two closest to the impacted area were influenced at the same rate. FA further suggested that the main factor regulating community structure is still natural disturbances, followed by human impact. Despite the detected changes and observed alterations produced by human activity in the area, the Praia Rasa shore is still unpolluted.

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**GROWTH OF AN ANTARCTIC MESODINIUM RUBRUM**

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We obtained the photosynthetic ciliate *Mesodinium rubrum/Myrionecta rubra* from an enrichment culture of sea-ice/water collected in January 1996 from McMurdo Sound, Antarctica. Since then, the ciliate has been grown in F/2 (20-30 psu) at 2.0 to 6.0 °C under 10-100  $\mu\text{moles photons m}^{-2} \text{ s}^{-1}$  irradiance. Under these conditions, *Mesodinium* reaches high densities ( $> 15,000 \text{ cells ml}^{-1}$ ) only when periodically supplied with a polar cryptophyte as prey. A 20-day experiment was done to determine how feeding affects *Mesodinium*; cell numbers and attributes were quantified by flow cytometry and epifluorescence microscopy. After addition of cryptophytes on day 1, cryptophyte numbers were reduced by approx. 80% in 48 hours. Red and orange fluorescence per *Mesodinium* cell increased 1.5 fold during this period and remained constant for 14 days. Treatments with cryptophytes exhibited a higher sustained growth rate (0.19 divisions day<sup>-1</sup>) than treatments without cryptophytes (0.09 divisions day<sup>-1</sup>). After 4 to 6 days the forward scatter (indicator of size) decreased 20% for the "fed" cells and remained lower than the control treatment ("unfed" cells) for the remainder of the experiment. On day 14, more cryptophytes (10,000 cells ml<sup>-1</sup>) were introduced to the previously "fed" treatment and it was monitored for an additional six days. Once again the cryptophytes were substantially reduced within 24 hours and average fluorescence per *Mesodinium* cell slightly increased. These data suggest that *Mesodinium* periodically obtain plastids from cryptophyte prey rather than permanently maintaining a reduced algal endosymbiont. These data also suggest that exposure to cryptophytes is necessary for sustained rapid growth of this photosynthetic ciliate.

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**SPATIAL AND TEMPORAL VARIABILITY IN THE ABUNDANCE OF CORAL REEF MACROALGAE IN THE FLORIDA KEYS**

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There is increasing concern over what appears to be a major decline in coral abundance and health in the Florida Keys. Frequently, this decline has been attributed to increased cover of macroalgae, which may be able to overgrow corals. However, there are few historical data which can be used to support or refute claims of major changes in the reef algal community and

limited understanding of spatial and temporal variability in macroalgae abundance. The first attempt at a synoptic "snapshot" view of benthic macroalgal abundance along the Florida Reef Tract was made in 1995, as part of a multi-disciplinary Keys-wide cruise. Twenty coral reefs were sampled with photogrammetric techniques. The coral reef algal community was dominated by several species of *Dictyota* and *Halimeda*. There was a large amount of inter-reef variability in macroalgal abundance. More intensive sampling of permanent photo-quadrats at Molasses Reef, off Key Largo, Florida, indicated substantial temporal variability, including large differences among seasons, years, and decades (1985 to 1988 vs. 1995 to 1996). The immediate causes for these large differences in spatial and temporal variability are unknown, although both "bottom-up" (nutrients) and "top-down" (herbivory) controls are probably involved and have been altered by anthropogenic activities. These activities may result in further changes in community structure of this reef system, with serious impact on ecosystem structure and function.

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#### **PHYLOGENETIC RELATIONSHIPS WITHIN THE RHODOPHYTA: THE UTILITY OF NUCLEAR LARGE-SUBUNIT RIBOSOMAL DNA SEQUENCE DATA**

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As a tool for providing enhanced resolution in red algal molecular phylogenies, the utility of data generated from nuclear large-subunit (LSU) ribosomal DNA sequences has not been adequately assessed. Prior work on other algal groups (e.g. the Dinophyta) has illustrated that within the LSU there are twelve divergent domains. It is hypothesized that the increased variation in these domains may provide enhanced resolution at equivocal nodes within phylogenies inferred from more conserved coding regions (e.g. the SSU). We have determined the LSU sequence for a number of red algae spanning the majority of rhodophyte higher-level lineages. These data will be used to establish the utility of the LSU in assessing phylogenetic relationships within the Rhodophyta and perhaps clarify evolutionary relationships left inconclusive from other molecular data.

### 54

#### **ECOLOGICAL IMPACTS OF DIFFERENT FORMS OF NITROGEN ON ESTUARINE PHYTOPLANKTON COMMUNITIES**

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"Nitrogen is nitrogen" is a common assumption when relating nutrient inputs to structural and functional alterations of natural phytoplankton communities. However, nitrogen (N) inputs can come from many sources which supply chemically distinct N compounds, including nitrate and ammonium. Since these different compounds possess different bioreactivities, they may lead to physiologically distinct responses in phytoplankton communities. The Neuse River Estuary, NC is a chronically N-limited system experiencing nutrient-driven eutrophication. N loading to the estuary is currently increasing with the relative proportion of ammonium growing. To determine if this compositional shift in N supply causes a corresponding compositional (and accompanying functional) shift in the local phytoplankton community, we performed small-scale (4 liter) manipulative bioassays. Community responses (primary productivity, biomass, algal composition) to nitrate and ammonium additions were measured over 48 hours. Results show that the phytoplankton community is N-limited. Preliminary analysis shows no significant difference between N types. In turbid environments, however, ambient irradiance may additionally affect N uptake and utilization. Nitrate assimilation requires more energy than ammonium assimilation. At high light levels, the difference in energetic cost is relatively small and may not significantly affect the use of one N compound over another. At lower light levels, however, energetic costs are more critical and the difference may cause a preference for ammonium over other N compounds. Investigations are underway to examine the interplay of low light levels with different N sources and the consequent impacts on phytoplankton community dynamics.

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#### **MULTIMEDIA PHYCOLOGY: DEVELOPING EFFECTIVE USES OF A DYNAMIC TEACHING TOOL**

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People retain only 20% of what they see and 30% of what they hear, but they remember as much as 80% of what they see, hear and "do" simultaneously. Multimedia uses computers to present and combine text,

graphics, audio, and video to promote students' learning by providing them a format to "see, hear and do simultaneously." We are developing a series of multimedia programs to teach phycology by using current research about the design of multimedia educational tools. The multimedia programs are intended to provide self-paced instruction and exploration by individual students, but can also be projected in lecture and laboratory. The first program to be completed focuses on the algal class Bacillariophyceae, commonly known as diatoms. After using the diatom program in two phycology classes, students were surveyed and interviewed to determine if their learning experience was enhanced. Students found that after observing modified images which emphasized frustule structures in the multimedia program, they took less time to find and observe these structures in laboratory samples. They also were able to visualize processes such as life cycles and reproduction better by studying the program's animations and video. Students' comments indicate they prefer a course that utilizes multimedia lessons in addition to traditional lecture. Through the use of multimedia, our goal is to stimulate greater student interest in algae, as well as increase learning efficiency and student satisfaction.

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#### **EFFECTS OF ATRAZINE AND ITS METABOLITES ON FRESHWATER ALGAE**

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Despite the presence of the herbicide atrazine and its metabolites in surface waters throughout the agricultural midwestern United States, little is known of the toxicity of the metabolites to non-target organisms such as algae. The acute toxicities of atrazine and its primary metabolites (desethyl- and disopropylatrazine) to eight species of freshwater algae representing both diatom and chlorophyte taxa were determined in the laboratory. Growth responses were monitored by fluorescence every two days for 15 days. Disopropylatrazine was approximately 25 to 400 times less toxic than the parent compound, whereas desethylatrazine was 12 to 80 times less toxic to several of the algae tested. The pattern of differential toxicity among algal taxa was similar for the metabolites and parent compound in that diatoms were generally less sensitive than the green algae assayed. Thus, effective concentrations of atrazine metabolites are well above those typically found in surface waters, however the effects of long-term exposures to

environmentally realistic levels and the potential for additive effects with the parent compound and other herbicides remain unknown. The differential species response to these commonly occurring herbicides indicates that shifts in algal community composition may be a predicted outcome of exposure to atrazine.

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#### **PHYLOGENETIC SYSTEMATICS AND BIOGEOGRAPHY OF THE GIGARTINACEAE BASED ON *rbcL* SEQUENCE ANALYSIS AND MORPHOLOGICAL EVIDENCE.**

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Recent molecular and morphological studies largely confirm the systematic conclusions presented earlier (Hommersand *et al.*, 1993, *Hydrobiologia* Vol. 260/261; 1994, *Botanica Marina* Vol. 37). We recognize the following: (1) a *Chondracanthus* clade, with two groups, one occurring primarily in the Western Pacific and by eastward distribution in the Atlantic Ocean, and a second occurring primarily along the coast of South and North America and by westward distribution in the Atlantic Ocean; (2) a *Gigartina* clade consisting of paraphyletic taxa *Rhodoglossum* and *Gigartina radula*, and *Gigartina* occurring in cool temperate regions of New Zealand, Australia and South Africa, with one species in Western Europe; (3) a *Sarcothalia* clade with one genus, *Sarcothalia*, distributed widely in the Southern Ocean; (4) a *Chondrus* clade consisting of the paraphyletic taxa *Iridaea*, *Chondrus canaliculatus*, and *Mazzaella* distributed primarily along the coasts of South and North America, and *Chondrus* found in the western North Pacific with one species in the North Atlantic Ocean; and (5) three undescribed monotypic genera: one based on *Gigartina alveata* from New Zealand, one on a plant from Tasmania (*G. Kraft*), and one on *Gigartina skottsbergii* from southern South America and Antarctica. Clades identified in *rbcL* trees correspond to taxa circumscribed on the basis of cystocarp anatomy, particularly the structural relationship between developing gonimoblasts and surrounding gametophytic nutritive filaments. Tetrasporangial characters, while often useful for recognizing genera, are less reliable as indicators of taxonomic affinity. Each clade contains a range of similar morphological types adapted to particular environments and gross morphology is useful

taxonomically primarily at species and infraspecific levels.

**58**

**SIZE SCALING IN MOTILITY OF COLONIAL VOLVOCALEAN ALGAE**

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We studied motility in *Pandorina morum*, *Yamagishiella unicocca*, *Volvox rousseletii* and *Volvox carteri* to determine the relationship between colony size and swimming speed. Each alga was videotaped as it swam towards an artificial light source. For each species, the average velocity remained fairly constant over a variety of light intensities examined. At the low Reynolds numbers typical for microorganisms moving through water, resistance is almost completely due to viscous drag. Stokes' law predicts that a sphere being pulled through the water experiences viscous drag that is directly proportional to both the velocity and the radius. However, the number of cells at the surface of a spherical colony should vary as the square of the colonial radius. The propulsive force that must overcome the drag is presumably correlated with cell number. Since an increase in radius relates to an exponential increase in cell number but only a linear increase in drag, we expect that species with larger colonial diameters will swim faster. *P. morum*, *Y. unicocca*, *V. rousseletii* and *V. carteri* had average diameters of 0.034, 0.040, 0.18 and 0.24 mm respectively and rates of progression of 0.27, 0.26, 0.46, 0.54 mm sec<sup>-1</sup>. respectively. Thus, size is positively correlated with increased swimming speeds, but the differences are not as great as would be expected from Stokes' law alone.

**59**

**A SYSTEMATIC STUDY OF SPECIES OF CHONDRACANTHUS (RHODOPHYTA, GIGARTINALES) FROM PACIFIC NORTH AMERICA**

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Twelve species are recognized for *Chondracanthus* from Pacific North America: six from the Pacific Ocean (*C. canaliculatus*, *C. corymbiferus*, *C. exasperatus*, *C.*

*harveyanus*, *C. spinosus*, *C. tepidus*) and seven from the Gulf of California (*C. acicularis*, *C. intermedius*, *C. johnstonii*, *C. macdougalii*, *C. pectinatus*, *C. teedii*, *C. tepidus*). Nearly ten additional taxa have been described under Gigartina, but these are traditionally treated as synonyms. Sequence analysis of the rbcL gene (RUBISCO) and ITS (Internally Transcribed Spacer) 1, 2, and 5.8S regions, combined with a morphological study of vegetative and reproductive structures were employed to investigate phylogenetic relationships among the species. Eighty sequences were obtained of the rbcL and ITS regions, representing geographically widespread populations. The phylogenetic trees inferred from the two molecular data sets were congruent. Ecads of *C. exasperatus* were unresolvable and sister to the *C. spinosus* complex. Sequences of *C. acicularis* (distinct from *C. acicularis* from Europe), *C. johnstonii*, *C. macdougalii*, and *C. pectinatus* from the Gulf of California were identical. Reinvestigation of these four species showed that they were continuously variable in their morphology. *Chondracanthus intermedius* from the Gulf equated to *C. intermedius* from Japan. *Chondracanthus corymbiferus*, *C. harveyanus* and *C. canaliculatus* were strongly supported taxa. External and internal vegetative and reproductive morphology, ecology, and texture were used to characterize species. Our data supports the continued recognition of the Pacific taxa listed above, but only two of those recorded for the Gulf of California.

**60**

**WINTER FLAGELLATE COMMUNITIES IN DIFFERENT TYPES OF SEA ICE IN THE ROSS SEA, ANTARCTICA**

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Sea ice plays an important role in modifying ecosystem structure in the all ice covered sea regions. Ice cover does not only offer a platform for animals to migrate, feed and reproduce on but it may also serve as a food source or a refuge for a variety of pelagic organisms. Sea ice supports highly diverse, often diatom dominated microbial communities, where both photo- and heterotrophic nanoflagellates (e.g. crypto-, prasino-, and haptophytes, dino- and choanoflagellates) are commonly encountered. The winter survival of pelagial (under-ice) consumers, e.g. Antarctic krill *Euphausia superba* is largely dependent on the availability (quality and quantity) of food items living associated with sea ice. This, in turn, is much related to the structure of sea ice. This presentation highlights the first results of a study that was made in the Ross Sea (Antarctica) in

May-June '98 and which aimed to relate observed differences in microbial community structure to different ice types (frazil, columnar etc.), and stages of new ice formation (grease, nilas and pancake ice), with a particular emphasis on flagellate diversity.

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**HIS-ASP SIGNAL TRANSDUCTION IN CHLOROPLASTS OF *HETEROSIGMA CARTERAE***

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Gene expression in chloroplasts may be controlled at each step, from transcript initiation through post-translational polypeptide modification. Cyanobacteria use signal transduction pathways involving His-Asp phosphorelay signaling, often between "sensor kinase" and cognate DNA-binding "response regulator" proteins, to regulate transcription. This His-Asp system has yet to be demonstrated functionally in chloroplasts. A putative chloroplast-encoded His-Asp transcriptional response regulator gene (*trg1*) has been identified in the estuarine raphidophycean alga *Heterosigma carterae*, as well as in several rhodophytes, and a glaucophyte. The *Heterosigma* *trg1* open reading frame is approximately 0.7 kb in length, contains no introns, and yields a conceptual translation product of 231 amino acids (26.8 kDa). Homology searches suggest that *Heterosigma* *trg1* has an *ompR*-like identity within the DNA-binding His-Asp response regulators. Amino acid residues conserved among all *ompR*-like response regulators are identical in *trg1*. Quantitative competitive RT-PCR showed that *Heterosigma* *trg1* is expressed at low levels (5  $\mu\text{g g}^{-1}$  total RNA). In contrast, *psbA* (positive control) transcript is abundant (60  $\text{mg g}^{-1}$  total RNA). Cell cycle analysis showed that *psbA* abundance oscillates in response to light (as has been previously demonstrated by both northern analysis and run-on transcription assays in *Heterosigma*). *Trg1* mRNA levels, however, were invariant. We hypothesize that a His-Asp phosphorelay mechanism may affect chloroplast genome transcription in a manner similar to bacterial signal transduction pathways in which "sensor kinase" and cognate "response regulator" proteins interact.

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**PHOTOPHYSIOLOGY & ENVIRONMENTAL CONDITIONS DURING A TOXIC BLOOM OF *CHRYSOCHROMULINA LEADBEATERI* (PRYMNESIOPHYCEAE).**

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A toxic phytoplankton bloom, dominated by *Chrysochromulina leadbeateri*, developed in the Ofotfjord-Tysfjord area in North Norway (68 °N) in the middle of May and ended in the end of June 1991 in the Vestfjord and adjacent fjord areas. The bloom was transported with outflowing low-salinity water along the Lofoten Islands where it caused losses of about 750 metric tons reared fish, mainly Atlantic salmon. A significant amount of chlorophyll *c*<sub>3</sub> was present in the water masses and yielded high in vivo absorption and fluorescence excitation signals at 470 and 585 to 590 nm, respectively. Chlorophyll *c*<sub>3</sub>-containing species comprises some of the most common bloom-forming and harmful phytoplankton species in European waters, i.e. species from the genera *Chrysochromulina*, *Prymnesium*, *Phaeocystis*, *Gyrodinium* and *Gymnodinium*. Light and electron microscopy verified that *C. leadbeateri* often was accompanied with other *Chrysochromulina* species and peridinin-containing dinoflagellates. Bio-optical characteristics, pigmentation, cell chemistry and growth rates of an isolate of *C. leadbeateri* obtained from this bloom, grown under high- and low-light conditions in laboratory, are given for comparison with field data consisting of spectral irradiance, salinity, temperature, [nitrate], [phosphate], [silicate], [chlorophyll *a*], [oxygen], light stimulated *in situ* fluorescence, bio-optics, pigmentation, and species composition/numbers. A possible scenario for the development of this bloom was the enhanced nutrient concentration during the winter 1990-91 in the Ofotfjord due to overwintering of approximately 1 million tons of herring from 0-250 m depth which may have sustained a large stock of mixotrophic *C. leadbeateri* in early spring.

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**PARTHENOSPORES IN *OEDOCLADIUM* (OEDOGONIALES, CHLOROPHYTA)**

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In Oedogoniales, parthenospores develop as thick walled spores from non fertilized egg cells. These spores, as well as oospores (sexual in origin), are usually deep

orange, with a high content of starch and oil. Parthenospores had been registered only in *Oedogonium* and *Bulbochaete*. Working on cultures from Argentine material of *Oedocladium carolinianum*, it was observed that oogonia may form two kind of spores: spores similar to oospores (most frequent), and spores with atypical features resembling oospores of *Oedocladium protonema* Stahl, the type species. In the present work, it is experimentally demonstrated that the unusual type of spores in *Oedocladium carolinianum* (two strains) and *Oedocladium cirratum* are parthenospores. Their morphology as well as some germination details are described. Evidence of cell polarity in egg cells is presented. Finally, taxonomic significance of these observations is discussed.

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#### **ECOPHYSIOLOGICAL INFLUENCES ON DINOFLAGELLATE TRAJECTORIES**

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The motility of dinoflagellates, a group responsible for many Harmful Algal Blooms (HABs), often is implicated in the formation of species specific cell aggregations, but information on cell trajectories under natural conditions is limited. Swimming speed, which together with orientation defines a cell's velocity, varies with cell size and with changes in environmental variables like light intensity, temperature and salinity. The even more obscure orientation component of velocity traditionally is attributed to geotaxis or phototaxis, but accumulating evidence suggests that a cell's biochemical state as determined by cell division and subsequent light and nutrient exposure may influence the strength and possibly the sign of these taxes. For example, one laboratory study with *Gymnodinium breve* suggests that the strength of negative geotaxis increases as cellular lipid concentration decreases, while another laboratory study with *Heterocapsa illdefina* suggests that the strength of negative geotaxis increases as cell diameter decreases. Mathematical models based on the principles of adaptive behavior provide a test bed for these laboratory results in the context of light and nutrient availability representative of field conditions. Currently, these behavioral models monitor the biochemical status of individual cells or subpopulations and combine information on internal cellular state with the stimulus provided by external environmental cues to determine

cell orientation. Together, these laboratory and modeling approaches support the formulation of new hypotheses that will be tested on natural populations in planned field campaigns like the ECOHAB:Florida program. Eventually, the field results will support the formulation of a realistic physical context for the behavioral models.

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#### **A COMPARISON OF SHALLOW AND MID-DEPTH BENTHIC MACROALGAL COMMUNITIES ON A CARIBBEAN CORAL REEF**

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Two benthic coral reef macroalgal communities were investigated in the vicinity of Boca Paila, Sian Ka'an Biosphere Reserve, Quintana Roo, Mexico. The well-developed reef system may be described as fringing or a small barrier reef. An assessment of the species composition of benthic macroalgae in a patch reef field (approximately 4.5 meter depth) and mid-reef spur and groove area (approximately 18 meter depth) was conducted by nondestructive photogrammetric methods utilizing a modified line transect. Cover, densities and frequencies of benthic macroalgal species from the two zones were compared. Species richness and diversity indices are provided along with a taxonomic checklist.

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#### **A 20-YEAR STUDY OF ASCOPHYLLUM NODOSUM POPULATION DYNAMICS NEAR A HEATED EFFLUENT IN EASTERN LONG ISLAND SOUND.**

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*Ascophyllum nodosum* populations exhibit easily quantifiable responses to changes in water temperature, making population studies a useful biomonitoring tool for assessing effects from heated effluents. Growth and mortality characteristics of four tagged populations (two thermally impacted, and two unimpacted) in the vicinity of Millstone Nuclear Power Station (MNPS) have been monitored over a 20-year period (1979 to 98) as part of a comprehensive ecological monitoring program. A Gompertz growth curve was fitted to monthly *Ascophyllum* tip length data using non-linear regression methods. Parameters derived from this function were

used to describe and statistically compare annual growth and growth rates. Mortality, or thallus breakage, was assessed by examining tag loss patterns. During exposure to moderate thermal addition (< 5 °C above ambient), the growing season began earlier and growth over the year was significantly higher than at reference sites farther away. Higher levels of thermal input reduced growth rates when temperatures reached 27 °C (7 to 9 °C above ambient), and total population mortality was observed when temperatures exceeded 30 °C (> 10 °C above ambient). Aside from this mortality event, no relationship between power plant operations and mortality was found during years of sublethal impacts. Rather, mortality was associated with natural environmental stress from storm-driven waves, or in several instances, with human intervention (vandalism).

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**FERTILIZATION IN *AGLAOTHAMNION OOSUMIENSE* (CERAMIACEAE, RHODOPHYTA) MONITORED BY THE USE OF FITC-LECTINS**

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The binding of fluorescein isothiocyanate (FITC) conjugated lectins to gametes of *Aglaothamnion oosumiense* 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. Four different lectins, concanavalin A (ConA), Soybean agglutinin (SBA), Dolichos biflorus agglutinin (DBA) and wheat germ agglutinin (WGA) bound to the surface of spermatia, but each lectin labelled different region of the spermatium. SBA and DBA bound only to the spermatial appendages but ConA bound to all the spermatial surface except spermatial appendages. WGA labelled narrow region which connects spermatial body and appendages. During the fertilization, the 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 or ConA, while trichogyne receptors were blocked by the complementary carbohydrates, D-glucose or N-acetyl D-galactosamin, respectively. WGA and DBA as well as their complementary carbohydrates had little effect on the gamete binding. The inhibitory effects of ConA and SBA was much more enhanced when two lectins were applied at the same time. The inhibitory effects of both

lectins were partially reversed (to 80 to 90% of controls) by addition of complementary carbohydrates.

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**USING SUPA TO CHARACTERIZE PHOTOSYNTHETIC PROCESSES OF HABS**

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During *in situ* deployments of the Self-contained, Underwater Photosynthesis Apparatus (SUPA), net carbon and oxygen productivity of contained phytoplankton samples were measured on minute time scales for up to 48 hours. The samples were contained within one-liter quartz chambers providing a means to track the responses of known populations. Blooms (or cultures) provided productivity signals that were optimal for short period measurements because of the highly concentrated samples. The results to be presented will contrast the productivity differences between two phytoplankton species, one representing HAB species (*Gymnodinium breve*) and one representing non-HAB, high biomass, species (*Thalassiosira pseudonana*). *Gymnodinium breve* produces blooms in the surface layer of the Gulf of Mexico and the Southeastern U.S. Atlantic coast. In these regions this surface dwelling species is exposed to intense irradiance during summer and therefore was expected to cope with experimental exposures in surface waters of the West Florida coast. *Thalassiosira pseudonana* had been used successfully in a number of laboratory and field studies with SUPA, several in the Gulf of Mexico. Under high natural irradiance conditions in SUPA, *G. breve*'s net productivity declined rapidly and showed no acclimation response over the course of the deployment. In contrast, *T. pseudonana* showed only moderate decline in net productivity under high irradiance, recovered rapidly when irradiance declined and showed an acclimation response. A better understanding of the response of *G. breve* to fluctuating, high irradiance exposure will help describe the processes leading to bloom formation, persistence and demise.

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**EFFECTS OF *DESMARESTIA VIRIDIS* ON SUBTIDAL COMMUNITY STRUCTURE: INHIBITION OF URCHIN MOVEMENT AND ALGAL RECRUITMENT**

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Experimental manipulations were used to evaluate the effect of *Desmarestia viridis* on community structure on subtidal pinnacles at Shemya Island in the western Aleutian archipelago. Pinnacle tops had dense, annual algal-dominated kelp stands, whereas the sides and bottoms had little to no foliose cover. In contrast, sea urchin densities were significantly higher on the bottoms and sides of pinnacles, and were seasonally variable on the tops. Highest urchin densities on the tops of pinnacles occurred in the fall as algal cover declined. In the summer, removal of various combinations of macroalgae from the pinnacle tops resulted in an increase in urchin density when *D. viridis* was excluded. As urchins moved to the tops from the sides and bottoms, after *D. viridis* was removed, the urchins were capable of eliminating the rest of the foliose algal cover. Artificial structures, which imitated *D. viridis*, were placed on pinnacle tops and inhibited urchin movement. This inhibition of urchin movement by *D. viridis* allows kelp stands to persist through the year on pinnacle tops. Although *D. viridis* has a positive effect on the entire kelp stand by protecting it from the negative effects of grazing urchins, it also had a negative effect on other foliose algae through competition for space. Removal of *D. viridis* and urchins from pinnacles resulted in an increase of other algal species. However, without *D. viridis*, the kelp stands would not be able to persist over the years in the presence of urchins.

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### EVOLUTION OF SECTIONS IN *HALIMEDA* IS LINKED TO TETHYAN-PANAMANIAN CLOSURE EVENTS.

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Partial 18SrDNA sequences, including a 102 base pair insertion, and the complete ITS region were used to infer phylogenies among 60 samples comprising all sections in *Halimeda*. Results show linkage with Tethyan or Panamanian closure events that separated the tropical Indo-Pacific and Atlantic. Section *Rhipsalis* is monophyletic; its basal dichotomy separates Indo-Pacific and Atlantic taxa. Subsequently, similar morphologies evolved independently in both ocean systems. Atlantic *H. monile* and Pacific *H. cylindracea* are morphologically similar but are not sister taxa. Section *Halimeda* is paraphyletic. Sister species *H. lacrimosa* and *H. gracilis* differ from all other representatives.

The remainder separates in an Atlantic clade with sister taxa *H. tuna* and *H. discoidea* and a Pacific clade also with *H. discoidea*. Thus, *H. discoidea* is paraphyletic. Amphi-Pacific samples of *H. discoidea* are closely related and so are amphi-Atlantic ones indicating that these populations disperse easily within an ocean system. Sections *Opuntia* and *Micronesicae* together form a clade. All species therein are monophyletic. Samples of *H. opuntia* from the Atlantic and Indo-West Pacific are found in sister clades with Pacific *H. fragilis* as next nearest neighbor. The Atlantic clade with *H. goreauii* and *H. copiosa* is sister to similar Pacific species. The link of the *Halimeda* phylogeny to vicariant events suggests that the extant taxa in this genus diversified ca. 20 million years ago. The fossil record dates back well into the Cretaceous. The earlier history could be due to several cycles of adaptive radiation and near-extinction.

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### LABORATORY OBSERVATIONS OF IMMUNOSUPPRESSION BY CYANO-BACTERIA

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Based on public health agency observations and previous research, environmental cyanobacteria can elevate populations of other, similar classes of organisms consisting of molds and fungi. While incidence may be due to factors providing cyanobacteria necessary minerals and dissolved organic matter, it is not our research subject. Our studies concentrate on undiagnosed, long term illnesses with potential for cyanobacteria as the causative vector. We continue to investigate a trend that normally non-pathogenic, resilient secondary infections could be the consequence of cyanobacteria. Focusing on identification of a narrow rift in immune defenses, our research reviews new and historically misidentified relationships. Our first goal is to bring about revised educational guidelines designed to replace referral of patients with legitimate, uninvestigated symptoms to the care of psychoanalysts. As our investigation shows, numeric incidence has been concealed by relegating symptoms of infection into categories restricting empirical investigation and masking recognition of cyanobacteria as a pathogen. The *Phycological Society of America* has provided invaluable culture methodology and studies enhancing identification of cyanobacteria. Continued research over five years has disclosed information into complex relationships cyanobacteria have with secondary mycoses. This finding should indicate a need for reduced diagnoses of neurologic disorders and to return

treatment to efficacious, mainstream medicine. The colonizing nature of cyanobacteria and affinities to secondary infections including black molds (*Aureobasidium*, *Bipolaris*), yeasts (*Hansenula*, *Candida*) and fungi (*Aspergillus*, *Penicillium*), have demonstrated a link with aflatoxin producing organisms likely to lead to the conclusion that all metastatic cancers spring from a single, spontaneous source. Our research continues investigation into health risks and potential serologic correlation.

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### **MOLECULAR AND MORPHOLOGICAL EVIDENCE FOR *BRYOPSIS* (CHLOROPHYTA) SYSTEMATICS**

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A phylogenetic analysis of sequences of the intron located in the chloroplast psbT gene from over 70 specimens of *Bryopsis* indicates that there are four well-resolved clades along the east coast of the United States. These clades differ significantly in either the seasonality or latitudinal distribution of their US members. Statistical analysis of nine morphological characters indicates that a significant portion of the variance in several of those characters can be attributed to differences among psbT clades. A unique collection from the Florida Keys included specimens belonging to two separate clades growing within a few meters of each other, but showing very distinct morphologies. They represent the equivalent of a common garden experiment and further support the hypothesis that there are genetically based morphological differences between these clades, but the differences are frequently eclipsed by the high level of phenotypic variation. The ecological and morphological data support the congruence of these molecular clades with species, but unfortunately the ranges of these characters are continuous. They cannot be used as fixed field characters and the morphological data from type specimens is insufficient to positively identify any of the groups. Preliminary data suggests that sequences from topotype specimens may be able to associate species names with psbT clades.

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### **ULTRASTRUCTURE AND SYSTEMATICS OF TWO NEW FRESHWATER CRYPTOMONADS, *PYRENOMONAS OVALIS* & *STOREATULA HANRATTYI* SP. NOV.**

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The ultrastructure and systematics of two new red colored freshwater cryptomonads, *Pyrenomonas* (= *Rhodomonas*) *ovalis* and *Storeatula hanrattyi* sp. nov., will be discussed. *Pyrenomonas ovalis* has the generic characteristics described for marine *Rhodomonas/Pyrenomonas* spp. It has a short furrow, a deep gullet, square inner periplast plates with beveled corners, a mucilagenous surface periplast component, a single chloroplast with two lobes connected by a pyrenoidal bridge, and a nucleomorph located in an indentation of the pyrenoid. *Storeatula*, which also has been described from marine waters only, has a single inner periplast sheet and a mucilaginous surface periplast component. Cells lack a furrow, they have a gullet, they have a bilobed chloroplast connected by a pyrenoid and they have a nucleomorph located in an indentation of the pyrenoid. Even though a furrow is lacking, there is a scalariform furrow plate that lines the gullet, suggesting that the gullet originated from a fusion of furrow folds. This freshwater *Storeatula* basically possesses the same features as the marine species but is designated as *S. hanrattyi* because of its freshwater occurrence.

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### **THE POSSIBLE ADAPTIVE ADVANTAGE OF CHLOROPLAST E. R. - THE ABILITY TO OUTCOMPETE AT LOW DISSOLVED CO<sub>2</sub> CONCENTRATIONS.**

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Algae with chloroplast endoplasmic reticulum apparently are able to grow at lower dissolved CO<sub>2</sub> concentrations than those eukaryotic algae without chloroplast E.R.. The dissolved CO<sub>2</sub> is low in marine waters (at normal pH 8 of seawater, HCO<sup>3-</sup> is 2000 μM while CO<sub>2</sub> is 15 μM at 25°C). Thus, algal blooms in open oceans usually consist of algae with chloroplast E.R. (usually diatoms, dinoflagellates, prymnesiophytes, raphidophytes or brown algae such as *Sargassum*). Anthropomorphic increases in atmospheric CO<sub>2</sub> should result in increased dissolved carbon dioxide in open oceans and an accompanying increase in eukaryotic algae that normally are growth limited by the low dissolved CO<sub>2</sub> (e.g. green and red algae). This increase in dissolved CO<sub>2</sub>, and decrease in algae with chloroplast E.R., could be beneficial to humans since this should

reduce toxin-producing algal blooms. In freshwaters dissolved CO<sub>2</sub> is more plentiful because of the lower pH of freshwaters (at pH 1 virtually all of the dissolved carbon is in the form of CO<sub>2</sub> and none as HCO<sup>3-</sup>). Eutrophic freshwaters are commonly dominated by eukaryotic green algae that lack chloroplast E.R. Under these conditions of relatively high dissolved CO<sub>2</sub>, algae with chloroplast E.R. would be at a disadvantage and only become abundant after a bloom of green algae or cyanobacteria had reduced the dissolved CO<sub>2</sub> concentration to a level where the green algae or cyanobacteria could not take up enough dissolved CO<sub>2</sub> to compete. Anthropomorphic activities have resulted in increased acidification of freshwaters with subsequent increases in green algal populations.

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#### **UTILIZING ALEXA 488 PHALLOIDIN TO VISUALIZE ACTIN MICROFILAMENTS IN ALGAE**

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Cytoskeletal actin microfilaments play a role in many processes in eucaryotic cells, including cytoplasmic streaming, morphogenesis, and organelle movement. Visualization of F (filamentous)-actin in algal cells usually has employed fluorescein isothiocyanate (FITC) phalloidin or rhodamine phalloidin. These may have several disadvantages, including rapid photobleaching and masking of actin by pigment autofluorescence. We demonstrate here that the new fluorescent probe Alexa 488 phalloidin from Molecular Probes, Inc. (Eugene, Oregon, USA) appears to be an effective probe for visualizing actin microfilaments in a variety of algal cells. This probe is very bright and quite photostable, and its emission spectrum minimizes interference from chlorophyll autofluorescence. We show actin microfilaments in nine green and one brown alga.

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#### **MECHANISMS THAT LINK THE CELL CYCLE TO THE DIEL CYCLE IN THE DINOFLAGELLATE, AMPHIDINIUM OPERCULATUM**

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Mechanisms which regulate growth and reproduction in dinoflagellates are critical to understanding how algal

blooms are formed. Cell division in dinoflagellates is frequently regulated by a circadian clock, with mitosis phased to the light/dark cycle. In this study, we sought to identify mechanisms which link the cell cycle to the diel cycle. The dinoflagellate *Amphidinium operculatum* is a useful model because its high division rate yields a nearly synchronous population. Using flow cytometry, we found that the cell cycle of *A. operculatum* is phased to the diel cycle, with S-phase beginning 7h and mitosis 15h after the onset of light. Alterations of the light/dark cycle showed that cell cycle progression is phased by the dark/light transition. In the more advanced alga, *Euglena*, cAMP dependent signaling is believed to mediate circadian rhythms. Therefore, we investigated the role of cAMP in circadian control of the cell cycle in *A. operculatum*. Measurement of intracellular cAMP by radioimmunoassay revealed that cAMP levels in *A. operculatum* varied on a diurnal basis, with minimum concentrations present at the onset of the light phase, and concentrations increasing to a maximum level, which occurred prior to the onset of dark. Furthermore, the cAMP phosphodiesterase inhibitor IBMX acts in a dose dependent manner to inhibit cell cycle progression at G1/S, indicating that cAMP signaling is involved either in the dark/light transition or is directly involved in regulating S-phase entry. Current research is directed at elucidating the precise role of cAMP signaling in the cell cycle of *A. operculatum*.

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#### **PHYSIOLOGICAL BASES OF CELL GROWTH IN PFIESTERIA PISCICIDA**

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The nutritional versatility of dinoflagellates is a complicating factor in identifying potential links between nutrient enrichment and the proliferation of harmful algal blooms. For example, although dinoflagellates associated with harmful algal blooms (e.g. red tides) generally are considered to be phototrophic and use inorganic nutrients such as nitrate or phosphate, many of these species also have pronounced heterotrophic capabilities either as osmotrophs or phagotrophs. Recently, the widespread occurrence of heterotrophic toxic dinoflagellates has been documented in turbid estuarine waters. One such species, *Pfiesteria piscicida*, has a relatively proficient

grazing ability compared to herbivorous protists of similar size, but also has an ability to function as a phototroph by acquiring chloroplasts from algal prey, a process termed kleptoplastidy. The physiological responses of *P. piscicida* to inorganic and organic nutrient additions were compared in strictly heterotrophic vs. kleptoplastidic cultures. When heterotrophic, *P. piscicida* responded to nutrients indirectly (i.e. its growth rate was correlated with increased phytoplankton prey abundance), although direct uptake of nutrients was detected. When kleptoplastidic, direct uptake of inorganic and organic nutrients was observed, and the uptake rates of certain substrates, such as urea, were relatively high compared to heterotrophic cultures. The results suggest that the response of *P. piscicida* to nutrient enrichment is complex, and that several pathways of nutrient stimulation may be ecologically relevant, depending on the relationship between nutrient quantity, nutrient quality, phytoplankton abundance, and *P. piscicida*'s nutritional state.

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### **MIXOTROPHIC MASS CULTURING OF CHLORELLA SPP. IN TAIWAN**

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The mixotrophic growth process has been used for the large-scale production of *Chlorella*, in Taiwan, for almost 40 years. The total production of *Chlorella* products, mainly spray-dry powders, in Taiwan, peaked to 1,400 tons per year in 1992. Production for 1997 was at the 1,000 tons per year level. Main pond construction, as a scale-up from inoculum culturing ponds, is most effectively done by using a proportional increase of pond area. This allows for a uniform increase in culture volumes. Mixing in the main culturing is done by energy saving double agitation systems. Suitable amounts of acetic acid, and nutrients are added to the basal media according to *Chlorella* cell growth stage. Specific considerations are the precautions taken to eliminate bacterial contaminants, mainly photosynthetic bacteria, by treatment through rotary moving Nylon-sheet filters, especially for an inoculum preparation. A standard practice is to treat the harvested cells with special steaming devices to destroy contaminated bacteria and stop chlorophyllase activities. The growing conditions in Taiwan easily allow for a growth rate  $10 \text{ g m}^{-2} \text{ day}^{-1}$  to be reached. Theoretically, under present day situation more than 2.0 L glacial acids are required to produce 1 kg *Chlorella* spray-dried

powders. In conclusion, we have successfully developed the large-scale production process for *Chlorella* using mixotrophic growth with glacial acetic acid as a C-source. Under our specific conditions of culturing, harvesting and products processing, Taiwan has become the world's greatest producer of *Chlorella* cells and products for health foods and specialty nutrition markets.

#### 79

### **PRELIMINARY PHYLOGENY OF THE MARINE RED ALGAL FAMILY DELESSERIAEAE (CERAMIALES, RHODO-PHYTA)**

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The Delesseriaceae currently contains the two subfamilies Delesserioideae and Nitophylloideae, encompassing 22 Groups/Tribes, about 95 genera and 310 species. The two subfamilies were primarily distinguished by Kylin (1924) based on pattern of apical growth, vegetative cell division, and procarp architecture. Uncertainties in many species in the interpretation of morphological features and convergent evolution in both vegetative and reproductive characteristics have led to taxonomic confusion at the generic and tribal levels. In this study, morphological characters used for grouping genera and tribes are examined and assessed in light of a molecular-based phylogeny inferred from sequence analysis of the chloroplast-encoded gene *rbcL*. So far, phylogenetic analyses support the distinction of the two subfamilies. *Sarcomenia delesserioides*, allied historically with both the Rhodomelaceae and Delesseriaceae, is found to cluster with the Dasyaceae.

#### 80

### **PHYLOGENETIC ANALYSIS OF *rbcL* SEQUENCE DATA FOR PACIFIC SPECIES OF PORPHYRA**

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The *rbcL* gene was sequenced in more than 20 species of *Porphyra* from Pacific waters (Alaska, British

Columbia, Chile and New Zealand). Relationships among the species were examined using PAUP (Phylogenetic Analysis Using Parsimony). Sequence data upheld some previously recognized relationships, such as *Porphyra abbotiae* and *Porphyra torta* being each other's closest relatives, but they also produced some surprising groupings. Collapse of the single most parsimonious tree after bootstrapping into two major polytomies suggests at least two major radiations have occurred within this group of species. The deeper polytomy includes the three distromatic species sampled (*Porphyra cuneiformis*, *Porphyra papenfussii* and *Porphyra schizophylla*). The present analysis supports the position of *Bangia* as the sister taxon to a holophyletic *Porphyra*.

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#### **INDUCTION OF CONCHOSPORE RELEASE: VARIATIONS ON A THEME IN NORTHEAST PACIFIC SPECIES OF PORPHYRA**

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Interest in *Porphyra* mariculture in Alaska and a proscription against importing non-indigenous species or stock prompted us to investigate conditions that stimulate release of conchospores in local species of interest. Many of these species had been investigated earlier by Waaland and colleagues using strains from Washington state. We observed at least one significant difference in release conditions in Alaskan strains of *Porphyra abbotiae*, *Porphyra fallax* and *Porphyra torta* compared to Washington strains. We have also produced blades from conchocelis of *Porphyra cuneiformis* and *Porphyra pseudolinearis*, although the latter less reliably than we would like. Monosporangia were observed on the conchocelis of most of these species under certain conditions. Our results confirm that species of *Porphyra* in this region have fairly specific requirements for release of conchospores and that important differences in these conditions can arise between populations within a region, perhaps eventually leading to speciation.

### 82

#### **PHYLOGENETIC ANALYSIS OF EUGLENOIDS USING 18S SSU RDNA.**

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The euglenoids are an ancient and extremely diverse lineage of eukaryotic flagellates with unclear intra- and inter-taxon relationships. Euglenoids are joined by unique symplesiomorphies, such as a surface pellicle and a closed mitosis with a series of separate subspindles. The taxonomy currently in use for the euglenoids is inconsistent with the available data and is in need of modification and revision. Most euglenoid phylogenies are largely intuitive reconstructions which are based on a limited number of morphological characters. Therefore, we have chosen to add molecular characters from the SSU rDNA to the morphological data to generate an overall phylogenetic framework for the euglenoids from which a taxonomy can be established. SSU rDNA sequences from photosynthetic, osmotrophic and phagotrophic euglenoids were aligned based on secondary structure, using the model of *Euglena gracilis* (Van de Peer, et al. 1997) as a guide. Phylogenetic analysis on the conserved areas of the sequence was performed using the programs PAUP\* 4.0d (Swofford, 1991) and TREECON 1.3b (Van de Peer, Y. and De Wachter, R. 1997). Trees derived using different criteria are in agreement. The euglenoids form a distinct monophyletic clade with phagotrophic members diverging prior to the phototrophic and osmotrophic members. Also, among the photosynthetic members, the biflagellate form diverged prior to the uniflagellate form. Additionally, the genus *Euglena* appears to be paraphyletic with osmotrophic taxa such as *Astasia* and *Khawkinia* falling out within the photosynthetic *Euglena* clade.

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#### **MICROPHOTOMETRIC ASSESSMENT OF SPECTRAL ABSORPTION AND ITS POTENTIAL APPLICATION FOR CHARACTERIZATION OF HARMFUL ALGAL SPECIES**

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Spectral absorption properties of phytoplankton have been used for both taxonomic and photo-physiological characterization and in support of bio-optical algorithms for estimating pigment concentrations and primary production. Conventional methods for the assessment of spectral absorption (measurements in suspensions or on samples concentrated on filter pads) can be used only to characterize bulk properties of natural populations,

thus making it difficult to identify contributions by individual species or taxa. Microphotometric methods allow the determination of spectral absorption properties of individual cells. As a compliment to existing cell-specific measurements (e.g., autoradiography, flow cytometry, etc.), this method enhances capabilities for probing the photo-physiology, growth and productivity of distinct taxonomic groups. Methods for microphotometric assessment of individual particle absorption spectra are described and compared to other techniques. Results with a variety of laboratory cultures, including the toxic dinoflagellate, *Gymnodinium breve*, will be presented. Factors influencing variability in spectral absorption, including pigment composition and 'package effects', are evaluated both within and among algal taxa. Potential applications of this technique for autoecological studies of harmful algal species in mixed populations will be discussed.

#### 84

#### **KARYOLOGY AND NUCLEAR GENOME QUANTIFICATION IN THE ORDER TRENTEPOHLIALES (CHLOROPHYTA)**

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Microspectrophotometry with the DNA-localizing fluorochrome DAPI was used to quantify nuclear DNA content in eight species representing three genera of the green algal order Trentepohliales (Chlorophyta). Comparisons of mean fluorescence intensity (If) values of algal nuclear genomes to those of chicken erythrocytes (RBC) resulted in an estimate of 1.1 to 4.1 pg for the algae. The diplobiontic life cycle of the Trentepohliales is not well documented, and it could not be determined if our non-reproductive samples were sporophyte or gametophyte, and thus no ploidy level was assigned. However, our genome quantification data show a clear doubling sequence in nuclear DNA content. This feature can be explained in one of two ways: the simplest explanation is that these isolates represent a mixture of generations, with both haploid gametophytes and diploid sporophytes. A more interesting hypothesis involves karyotype data: available data (recorded for 15 taxa) show a large scale variation in chromosome numbers (from [4]12 to 56) in the Trentepohliales. This range of chromosome numbers could represent a polyploid series. Our hypothesis

suggests that, if the isolates in our study represent the same phase, then the doubling sequence in nuclear DNA content could be the result of the variation in chromosome numbers, thus indicating that evolution in this group has involved polyploidy accompanied by doubling of genome size.

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#### **CONTINUOUS MITOCHONDRIAL LARGE SUBUNIT RIBOSOMAL RNAs IN CEPHALEUROS PARASITICUS KARSTEN (TRENTEPOHLIALES, CHLOROPHYTA): PHYLOGENETIC IMPLICATIONS**

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Both continuous and discontinuous mitochondrial large subunit ribosomal RNAs (mtLSU rRNA) have been reported among green algae. The phylogenetic distribution of these two types of mtLSU rRNA has been shown to be consistent with phylogenetic relationships previously suggested by both ultrastructural and nuclear rRNA sequences. Discontinuous mtLSU RNAs have only been found in zoosporic chlorophycean lineages with a clockwise flagellar apparatus configuration as well as their autosporic relatives, whereas continuous counterparts have been reported in all the investigated zoosporic green algae with a counterclockwise orientation as well as their autosporic descendants. Therefore, the type of mt rRNA can be used as an additional phylogenetic character in resolving conflicting inferences of relationships among green algal lineages. Trentepohliales provides an example. Its phylogenetic position raises questions: on the basis of the counterclockwise flagellar apparatus as well as on the phylogenetic analysis of nuclear-encoded rRNA gene sequences, this group is assigned to the Ulvophyceae (or to a phylogenetic position close to the Ulvophyceae), but phragmoplast type cell division in these algae is a major charophycean character. Our studies indicate the presence of continuous mtLSU rRNAs in *Cephaleuros parasiticus*, a result that is consistent with an ulvophycean affinity. More information on additional Trentepohliales as well as ulvophycean taxa is needed in order to confirm our suggestions.

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**PLASMID-LIKE DNA MOLECULES IN COENOCYtic GREEN ALGAE ARE CHLOROPLASTIC IN LOCATION AND IN ORIGIN**

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Plasmid-like molecules have been isolated from *Ernodesmis verticillata* and *Ventricaria ventricosa*. Molecular cloning of restriction fragments of these molecules has provided probes for analyzing these unusual molecules further. Fluorescence *in situ* hybridization demonstrates that the plasmids are exclusively located within chloroplasts. This finding would explain how plasmids are transmitted from the multinucleate phases to the uninucleate flagellated stages and back again. Whereas the chloroplast genomic DNA exists as nucleoids scattered throughout each plastid, the plasmids are arranged in a spherical configuration around the pyrenoid. The only homologies revealed from plasmid sequence analyses are short stretches of deduced amino acid sequences with high degrees of identity to protein components of photosystems I and II. Thus far, these include portions of psbA, psbB, psbC, psbF and psaB genes. The clone containing the psbB gene fragment also labels a discrete band in northern blots that corresponds in size to a band labeled with a psbB gene probe from *Chlamydomonas*. Surprisingly, neither the homologous nor heterologous probes label chloroplast genomic DNA in Southern blots, instead hybridizing solely to plasmid DNA. These data collectively suggest that green algal plasmids probably originated from chloroplast DNA. However, their genes for photosynthetic proteins have apparently diverged sufficiently to preclude their hybridization to genomic DNA. These findings raise interesting questions about the gene content and organization of chloroplast genomic DNA in these algae. Also it is presently unclear whether functional psa/b transcripts arise from chloroplast genomic DNA, plasmid DNA or both.

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**A NEW TECHNIQUE FOR THE EXTRACTION OF INTRACELLULAR INORGANIC NITROGEN OF MARINE MICROALGAE**

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The quantification of intracellular nitrate, nitrite and ammonium may provide important information on the

physiological state of cultures, as well as on the ability of species store nitrogen pools. Techniques for extracting intracellular inorganic nitrogen (IIN) show different degrees of efficiency, as a result of the kind of sample and the biochemical composition of the species. A new methodology for extracting IIN was tested with samples from different growth phases of *Dunaliella tertiolecta*, *Chlorella minutissima*, *Tetraselmis gracilis*, *Isochrysis galbana*, *Nannochloropsis oculata*, *Hillea* sp., *Skeletonema costatum*, *Phaeodactylum tricornutum*, *Synechococcus subsalsus* and *Prorocentrum minimum*. Samples of cultures (40 - 120 mL) must be filtered through fiberglass filters previously treated in muffle furnace at 400 C for four hours. The method includes immersion of samples in 30 mL of Milli-Q water (MQW) for 12 hours at 4 °C. After this period samples are immersed in 15 mL of hot MQW (70-80 C) for two minutes, followed by flux of small amounts of MQW through the samples/filters, provided by a vacuum pump. All volumes of water are gathered, filled to 100 mL and the IIN is measured by conventional methodologies for analysis of dissolved inorganic nutrients. The combination of three different treatments (osmotic shock, heating and flux of water through samples) seems to increase IIN extraction. Present results are at least 20% greater than the IIN values available in the literature, indicating high efficiency of the new technique.

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**SEASONAL FLUCTUATIONS IN TISSUE NITROGEN AND PHOSPHORUS OF MACROALGAE FROM ARARUAMA HYPERHALINE LAGOON, BRAZIL**

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Araruama Lagoon is a coastal environment in Southeastern Brazil, under a subtropical climate. Former studies demonstrated that local macroflora is composed by ninety-eight infrageneric algal taxa. N and P contents of five abundant species (one Chlorophyta and four Rhodophyta) were analyzed during an annual cycle. Except for *Gracilariopsis tenuifrons*, all species presented great variations in the N, P, and N:P (by atoms) values along time. Among the five species the N concentrations varied from 1.9% (*Ulva lactuca*, Spring) to 8.3% (*Acantophora spicifera*, Winter) of dry weight.

P contents ranged from 0.24 % (*G. tenuifrons*, Winter) to 0.71% (*Hypnea cornuta*, Fall) of dry weight. Results for N:P ratio indicated high values, ranging from 13.2 (*U. lactuca*, Spring) to 29.4 (*Gracilaria cervicornis*, Fall). The analysis of dissolved inorganic nutrients in the water showed low P concentrations, agreeing with predominance of high N:P ratios obtained for species. Although Araruama Lagoon shows characteristics of an eutrophic environment, increments of macroalgal biomass seem to be limited by phosphorus availability.

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**FOOD UPTAKE IN MIXOTROPHIC  
DINOFLAGELLATES USING MEMBRANE  
MARKERS**

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Evidence of widespread mixotrophy in dinoflagellates has been accumulating in recent years, with few data on the relative contribution of phototrophy and heterotrophy to the growth of the photosynthetic predator. An approach was sought that would allow detection and quantification of the feeding process especially in marine protists bearing chloroplasts, not only under controlled culture conditions but in field populations as well. A membrane marker technique used to study endocytosis in motor nerve terminals and in fertilized eggs has been applied to the study of phagocytosis in mixotrophic protists. Although the protocol is still in development, experiments with bacterized cultures of *Gymnodinium sanguineum* and *Prorocentrum micans* exposed to fluorescent styryl dyes have demonstrated that the probes are non-fluorescent when dissolved in seawater but fluoresce as soon as they attach to membranes, are completely non toxic for several days and become associated with cellular inclusions. Work is in progress to confirm the nature of the inclusions as food vacuoles. Because visual discrimination between photosynthetic pigments and probes is easily accomplished by epifluorescence microscopy, the technique appears promising in quantifying the fluorescent inclusions using image analysis software.

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**LARGE SCALE CULTURE OF MARINE  
PHOTOSYNTHETIC MICROALGAE FOR  
BIOMEDICINAL PRODUCTION**

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A large-scale culture system for marine microalgae was developed to reliably provide pharmaceutically promising metabolites in the quantity needed for chemical analyses, pharmacological evaluations and clinical testings. The outdoor system was built upon existing 15000-liter cylindrical tanks using enriched filtered coastal seawater. The enclosed cultures were gently stirred and aerated; solar radiation was supplemented with overhead lamps and an underwater very high output fluorescent unit. Temperature was maintained within 2 °C of the target with a submerged heating/cooling unit. Three clones were grown: *Amphidinium operculatum* var. *gibbosum*, a free-swimming dinoflagellate producing metabolites with antitumor activity; *Prorocentrum mexicanum*, a thecate benthic dinoflagellate; and *Pseudo-nitzschia multiseriis*, a boreal-temperate pennate diatom producing domoic acid. Cells in early stationary phase of growth were precipitated, the supernatant was siphoned off and the slurried precipitate was harvested by continuous centrifugation. *Amphidinium operculatum* var. *gibbosum* reached concentrations over 10 millions cells per liter, with a growth rate of 0.35 per day; however production of metabolites was low and cell leakage during harvest could not be excluded. Several attempts with *P. mexicanum* and *P. multiseriis* failed, mostly as a result of light limitation, pH fluctuations and the presence of contaminants passing through the filtration system. Culturing success appeared to heavily depend on the level of solar radiation received by the culture in the first 10 days, a condition which may jeopardize the use of the facility during the winter months at our latitude, unless additional artificial lighting is provided.

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**THE BIOALGICIDAL POTENTIAL OF FRESH  
CUT ALFALFA AND REED CANARY GRASS ON  
SEVERAL ALGAL SPECIES**

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The bioalgal properties of several forages and straws were examined as an alternative to the use of copper containing algicides. The algicidal effects of barley straw have previously been documented. However, the availability of barley straw can be a problem. In response to this problem, an algal screen was developed

to test other forages and straws that might also be effective bioalgaecides. Nine different forages and straws were tested on twelve algal species. The forages and straws that were assayed included barley, fescue, reed canary grass, alfalfa haylage, fresh cut alfalfa, rye, birdsfoot trefoil, wheat, and oats. The algal screen included *Anabaena*, *Ankistrodesmus*, *Euglena*, *Gloeocapsa*, *Navicula*, *Oscillatoria*, *Pithophora*, *Scenedesmus*, *Selenastrum*, *Spirogyra*, *Synechococcus*, and *Synedra*. The forages and straws, at a rate of 5g dry material L<sup>-1</sup>, were decomposed in barrels filled with water. The liquids from the decomposed plant material were then tested at monthly intervals for algicidal properties. Fresh cut alfalfa and reed canary grass showed the most promising results. Growth rates were significantly reduced for several of the target groups such as cyanobacteria and filamentous algae. Reductions of greater or equal to 50% of the control will be reported.

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### A MOLECULAR MARKER HIGHLY LINKED TO THE SEX GENE IN *GRACILARIA GRACILIS*

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Among red algae, sexual reproduction is widespread and its morphological and cytological bases have been well described (Hommersand and Fredericq 1990, for review). However, on a genetic level, there is still a great lack of information concerning sexual determination. For *Anthithamnion spirographidis*, Drew (1955) indicated that segregation of sex chromosomes during meiosis explains the equal numbers of male and female gametophytes. More recently, van de Meer (1990) suggested that the primary control of sex determination in *Gracilaria thikvahiae* is given by a single pair of alleles designated mtf (female mating type) and mtm (male mating type) rather than by sex chromosomes as it happens in some brown algae (Evans 1965, Yabu and Sambonsuga 1981). In this study, in order to search for a molecular marker linked to sex genes we used a modified version of Bulk Segregating Analysis, a tool normally applied to find molecular markers for disease resistance or susceptibility in plants of agricultural interest. This method, based on the

amplification of genomic DNA with arbitrary primers (Random Amplified Polymorphic DNA's, RAPD's) allowed to find one RAPD marker for male individuals of *Gracilaria gracilis*. The marker was linked to the male allele and, as expected, it was also present in diploid tetrasporophytes. The marker presence was tested on 59 gametophytic individuals (39 males and 20 females) germinated, *in situ*, from spores of a single tetrasporophyte. This segregation analysis showed almost 100% linkage of the RAPD marker to the male allele.

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### EXTINCTION AND *rbcL* RATE HETEROGENEITY AMONG MAJOR LINEAGES OF THE CHARACEAE (CHAROPHYCEAE)

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Charophytes are nonmarine green algae that are close relatives of land plants according to recent molecular and morphological studies. The oldest fossil fructifications (termed gyrogonites) of these algae are found in later Silurian-Early Devonian, after which the group experienced several phases of diversification and extinction. A distinct break occurred at the Cretaceous-Tertiary Boundary in charophyte diversity: this event was marked by the extinction of about fifty per cent of the taxa. The major difference between the Cretaceous and Tertiary charophyte floras is the disappearance of the Clavatoraceae family, which became extinct at the same time as the last dinosaurs at the end of the Cretaceous (Dinosaur eggshells have been found together with gyrogonites in several localities of the terminal Cretaceous). However the extinction crisis occurred during a diversification phase of one family, the Characeae, which is the only surviving family of charophytes. Molecular studies of the chloroplast-encoded Rubisco large subunit (*rbcL*) gene show large differences in sequence divergence within the six extant genera, in particular between lineages containing the two most species-rich genera, *Chara* and *Nitella*. We hypothesize either or both of the following processes may be responsible: (1) rates of *rbcL* evolution varied between genera, (2) extant species in the two groups represent diversifications from ancestors of very different ages.

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**MOLECULAR BIOLOGY OF ACYL CARRIER PROTEIN IN *CHAETOCEROS MUELLERI* AND *NITZSCHIA ALBA***

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Potential uses for triacylglycerols, storage products of diatoms, have made biosynthesis of these molecules a subject of increasing medical, industrial, and commercial interest. The ubiquitous acyl carrier protein, an essential protein in fatty acid biosynthesis, is highly conserved and a useful marker to study the molecular biology of lipid biosynthesis. Using Southern Blot Analysis, it was determined that two acyl carrier protein genes are present in the chloroplast genome in *Chaetoceros muelleri*, possibly within the inverted repeat characteristically present in diatom chloroplast genomes. In diatoms, several fatty acid biosynthetic genes are typically located in the chloroplast genome. However, little is known about lipid biosynthesis and the chloroplast genome of non-photosynthetic diatoms, such as *N. alba*. Fluorescent microscopy using DAPI stain indicates that a plastid genome is maintained in *N. alba*. However, total genomic DNA extraction and separation on a cesium chloride gradient yields predominantly nuclear DNA, suggesting that the plastid genome is greatly reduced. This suggests that the gene for acyl carrier protein and other fatty acid biosynthetic proteins may be encoded in the nucleus of *N. alba*. Southern Blot Analysis is being used to determine whether acyl carrier protein is nuclear or plastid encoded in *N. alba*. Preliminary ultrastructure results also indicate that *N. alba* has not retained typical chloroplast ultrastructure. Since fatty acid biosynthesis generally occurs in the chloroplast, immunoelectron microscopy is being utilized to compare subcellular localization of acyl carrier protein in *Chaetoceros* and *Nitzschia*.

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**GENETIC MODIFICATION IN *CHONDRUS CRISPUS* THROUGH PROTOPLAST FUSION AND MUTAGENESIS**

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Methods for producing protoplasts from red algal spores were recently developed. Spore-protoplasts are totipotent and exhibit increased viability and regeneration. Spore-protoplasts made from tetraspores were cultured to produce callus-like masses of filaments which were isolated, repeatedly subcultured and used in mutagenesis experiments to produce *Chondrus* plants with increased thermal tolerance. To date, UV-treated regenerants from filament cultures, followed by repeated selection, have survived 56 days at 28 °C (42 days longer than previously reported) versus 14 days for controls. Spore-protoplasts from both life-history phases of *Chondrus crispus* were utilized in fusion experiments to produce somatic hybrids. Successful fusions (44 plants) were microscopically identified, marked and transferred to bubbling flasks. Putative fusion products were initially analyzed using micro-infrared spectrophotometry. One plant (7/12#1), produced in a haploid+ diploid fusion experiment, had a spectrum similar to a gametophyte, except that the 805 cm<sup>-1</sup> peak, indicative of 3,6 anhydrogalactose-2-sulfate, was absent. Carrageenan analysis of 7/12#1 revealed a molar ratio of 1:0.5: 0.92 (galactose: 3,6 anhydrogalactose: sulfate), unlike a typical *Chondrus* gametophyte of 1: 0.8: 1.27. In addition, the DNA level of 7/12#1, as revealed by DAPI microspectrofluorometry, was 0.36 pg while gametophyte and tetrasporophyte controls were 0.23 and 0.47pg, respectively. This is the first report of aneuploid production and cell wall constituent alteration in a morphologically complex and commercially valuable seaweed and gives validity to the use of spore-protoplasts for fusion.

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**PHYLOGENY OF THE GREEN ALGAE: THE 28S rRNA GENE AND THE SPHAEROPLEALES**

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The green algal class Chlorophyceae has undergone numerous, recent taxonomic revisions. However, relationships between and within certain orders remain an open question. In particular, questions regarding the Sphaeropleales have not been resolved. Utilizing partial sequences (ca. 2200 bases/taxon) from the 28S gene of the nuclear-encoded rDNA gene array, the main objectives of this research were to test the monophyly of the genera within the family Sphaeropleaceae (sensu Mattox and Stewart), to examine relationships of taxa within the family, and to assess the position of the order

Sphaeropleales within the green algae. Analyses of the 28S data corroborated previous studies of the internal transcribed spacer (ITS) in rejecting monophyly of *Sphaeroplea soleirolii*, a taxon united by shared zygote wall morphology but exhibiting diversity in vegetative characters. The 28S data also were used to address questions regarding the enigmatic *Sphaeroplea fragilis*, which does not possess an apical cone in the distal fiber of the male gametes. The 28S data support ITS and ultrastructural data in placing *S. fragilis* as a basal member of the filamentous genus *Sphaeroplea*. Parallel data sets, from the 18S and 28S rRNA genes, comprising a sampling of organisms representative of the chlorophycean orders (*sensu* Mattox and Stewart) were compared. Both molecular data sets support a close alliance among *Sphaeroplea*, *Atractomorpha* and *Ankyra*. Although *Cylindrocapsa* has been allied with *Sphaeroplea* in one classification (*Sphaeropleaceae sensu* Mattox and Stewart), neither molecular data set supports *Cylindrocapsa* as a close ally of the group.

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**PATTERNS IN THE EVOLUTION OF SEXUALITY: THE CHLAMYDOMONAS EXAMPLE**

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*Chlamydomonas* is a unicellular member of the green algal class Chlorophyceae. Although more than 400 species of *Chlamydomonas* have been described, the sexuality of only a few is known in detail. Of these species with known life histories, considerable diversity in the form of sexuality has been described. While most are isogamous, a few species exhibit heterogamy (including anisogamy and oogamy). In addition, sexual species can be characterized as heterothallic or homothallic. Heterogamy and homothallism are traditionally regarded as derived conditions. These assumptions about the polarity of sexual characters were tested by phylogenetic analysis of DNA sequence data from the 18S rRNA gene. Genomic DNA from sexual isolates of *Chlamydomonas* served as templates for PCR amplification of the 18S rRNA gene. Amplified product was sequenced by automated methods. The sequence data were aligned and analyzed using computer-based phylogenetic methods. Results from phylogenetic analysis suggest that heterogamy and homothallism have evolved independently in several lineages. Within the Chlamydomonadales, homothallism appears to be the ancestral condition. These observations challenge traditional concepts of sexual character stability.

However, the diversity within the genus *Chlamydomonas* is consistent with higher level classifications of the green algae which implicitly support the notion that heterogamy has evolved independently in distinct families and orders.

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**STABILITY OF PSEUDO-NITZSCHIA (BACILLARIOPHYCEAE) SAMPLES IDENTIFIED USING SPECIES-SPECIFIC FLUORESCENT IN SITU HYBRIDIZATION**

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Some species within the genus *Pseudo-nitzschia* are associated with production of domoic acid, the agent responsible for amnesic shellfish poisoning (ASP). Identification and enumeration of particular *Pseudo-nitzschia*, especially in natural populations that frequently harbor multiple species, is difficult and time consuming because of a need for detailed morphological observations that often require scanning electron microscopy (SEM). We have developed species-specific large sub-unit ribosomal RNA (LSU rRNA)-targeted fluorescent DNA probes for a variety of *Pseudo-nitzschia* species. Probes are applied using whole cell hybridization and a custom filtration manifold, enabling rapid quantification of target species in field samples. A limitation of this method has been the perceived necessity for immediate sample processing, restricting the practicality of analyzing a large number of samples for target species. In this presentation we describe results of comparing a variety of fixatives and assessments of sample stability. Of the variety of fixatives tested an ethanol-based treatment gave the best results in terms of brightness and uniformity of probe fluorescence. Culture samples treated with this fixative and stored at either room temperature or -20 °C were stable for at least three weeks, showing little signal degradation. Likewise a field sample stored in the same fixative at -20 °C was also stable for at least three weeks. Post hybridization, samples stored at 4 °C were found to retain their fluorescence for approximately three weeks. These results indicate there is a wider window of opportunity for analyzing samples than we previously thought, allowing the accumulation of potentially interesting samples for later analysis.

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**IMPACT OF A RECURRENT COASTAL PLUME ON LAKE MICHIGAN PHYTOPLANKTON; A PRELIMINARY ASSESSMENT**

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A recurrent coastal plume (RCP) of resuspended materials occurs annually within southern Lake Michigan. This highly reflective, turbid plume appears to be initiated by a major late winter storm immediately after 'ice out'. The silt- and clay-like particles composing the RCP are thought to originate on the lake's western shore before transport to the resuspension site. The RCP may extend greater than 10 km in width and 300 km in length and eventually veers offshore along the eastern shoreline, coinciding with the area of highest measured sediment accumulation in the lake. In this introductory year of a 5 year, NSF-NOAA interdisciplinary program assessing this episodic event, we are evaluating the impact of the RCP on the lake's phytoplankton assemblages. Although both light and nutrient availability appear to regulate assemblages during the spring isothermal period, linkages between the RCP and phytoplankton growth and primary production have been postulated, but not verified. As such, the RCP provides a novel opportunity to examine the influence of light availability on phytoplankton rate processes at both the species and community-levels. During a series of spring cruises incorporating both process measurements and langrangian experiments, the physical, optical, and autotrophic components of the water column were characterized. Here, we summarize the impact of the RCP with respect to the light-limitation of natural populations. The impact is characterized using phytoplankton production, growth, light absorption, quantum yield, and biomass measurements.

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### PHYSIOLOGICAL RESPONSE TO UV-B RADIATION STRESS IN TWO MICROALGAE FROM THE COAST OF CHILE.

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The response to UV-B radiation was evaluated in two distinctly different phytoplankton species of the Pacific coast of South America. Various physiological parameters (specific growth rate, photoreactivation, UV-absorbing compounds, photochemical capacity, photosynthesis vs. irradiance, soluble proteins and Rubisco) were compared in order to assess whether the two species differ in their photoadaptational responses to UV radiation. A Magallanes isolate of the red tide dinoflagellate, *Alexandrium catenella*, and a central coast isolate of the diatom, *Phaeodactylum tricoratum*, were grown on a 16:8 photoperiod in polyethylene bags and f/2 medium. Exponential cultures, acclimated to 103  $\mu\text{mol m}^{-2}\text{s}^{-1}$  PAR and 4  $\text{Wm}^{-2}$  UV-A radiation, were exposed for 2 or 4 h daily to 0.11 to 1.31  $\text{Wm}^{-2}$  UV-B radiation provided by fluorescent UVB-313 lamps. Cultures were covered by optical filters to eliminate UV-B and/or shorter wavelengths. UV-A (365  $\pm$  12 nm) and UV-B (313  $\pm$  12 nm) radiation were measured with an interference filter radiometer. Although *A. catenella* synthesized considerable amounts of UV-absorbing compounds, whereas *P. tricoratum* did not, the dinoflagellate was far more sensitive to UV-B radiation than the diatom: a 50% reduction in growth was measured at 0.4  $\text{Wm}^{-2}$  (2 h d<sup>-1</sup>) for *A. catenella* and at 1.13  $\text{Wm}^{-2}$  (4 h d<sup>-1</sup>) for *P. tricoratum*. The higher resistance to UV-B radiation by *P. tricoratum* is consistent with measurements of photoreactivation suggesting higher rates of DNA repair compared to *A. catenella*. While our results point to important differences in the photoadaptational responses to UV-B radiation of these two organisms, they do not provide clues to explain the persistence of *A. catenella* blooms in high UV radiation environments.

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### COMPARATIVE MOLECULAR ANALYSES OF PORPHYRA PLASMIDS DEMONSTRATE INTEGRATION INTO ORGANELLAR DNA OF SOME SPECIES

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Conspicuous plasmids are visible in electrophoresed genomic DNA of many eukaryotes, including some red algae, but these are apparently absent in the DNA of closely related species. The present study explains this paradox for some species in the genus *Porphyra*: plasmid-homologous sequences can be cryptic because

they are integrated within organellar genomes. *Porphyra pulchra* plasmids Pp6859 or Pp6427 were used to screen Southern blots of other northeastern Pacific *Porphyra* species for homologous sequences. Pp6859 hybridized to high molecular weight DNA in *P. nereocystis* and *P. abbottae*. Pp6427 hybridized to high molecular weight DNA in *Bangia* sp. and *P. lanceolata*. Molecular characterization of clones isolated from *P. lanceolata* document the insertion of plasmid-homologous sequence (approximately 4300 base pairs) into the chloroplast genome. Through comparison to the published *P. purpurea* chloroplast genome (Genbank Accession #U38804) the site of insertion maps to the tRNA for isoleucine. Regions flanking the insertion are clearly identifiable and conserved. Many organisms lack introns in chloroplast genes for isoleucine tRNA, including *Spinacia oleracea*, *Picea abies*, *Chlamydomonas reinhardtii*, and *Euglena gracilis*. Other species contain introns in the isoleucine tRNA gene (*Porphyra purpurea*, *Marchantia polymorpha*, and *Zea mays*) and their introns begin in a similar position, but these are smaller (241 to 950 bp) and share no sequence similarity to the *P. lanceolata* plasmid-like insertion. Given that many *Porphyra* species maintain distinct high copy number plasmids, the presence of a substantial plasmid fragment in the plastid genome of *P. lanceolata* may indicate trapping of a moveable sequence element.

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### A PRELIMINARY ANALYSIS OF THE GROUP I INTRONS IN THE 18S RRNA GENE OF BANGIA (BANGIALES) RHODOPHYTA

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A large insertion was found near the 5' end (helix 21) of the 18S rRNA gene in a variety of global collections of the genus *Bangia*. Subsequent sequence analysis of this region displayed many characteristic sequences and structural features similar to the C1 subgroup of group I introns. RT-PCR (reverse transcriptase PCR) revealed that this insertion is spliced out during transcription. In addition, a previously reported Group I intron near the 3' end of the gene was also present in some collections of *Bangia*. In fact, the samples displayed three different configurations: those with both introns, those with only the intron near helix 21 or those with neither intron. Phylogenetic analysis of the two introns has revealed

similar trends to those of the 18s rRNA gene analysis for this genus. All freshwater collections were nearly identical and group consistently together in the phylogenetic trees. Marine collections form several well supported clades within Atlantic and Pacific basins. The presence of introns within other bangiophyte taxa will also be discussed.

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### PRODUCTION AND CHEMICAL ECOLOGY OF CYANOBACTERIAL METABOLITES

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Cyanobacteria are recognized as a rich source of toxins and other pharmacologically active compounds. Often chemical studies have focused on toxin production. However, cyanobacteria also appear to produce a wide assortment of unusual secondary metabolites for which little or no biological or biochemical role has been attributed. Ongoing research suggests that many of these metabolites may be ecologically important. For example, some of the metabolites produced by cyanobacteria appear to function as effective feeding deterrents to both vertebrate and invertebrate grazers. The production of cyanobacterial chemical defenses may, in some cases, actually facilitate the formation of cyanobacterial blooms. This appears to be especially true in the presence of grazers. In highly competitive environments, other metabolites may function to suppress bacterial and fungal overgrowth or act allelopathically to inhibit the growth of other cyanobacteria and algae.

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### ASSIMILATORY NITRATE REDUCTASE FROM THE RAPHIIDOPHYCEAE HETEROSIGMA AKASHIWO CONTAINS PROTOZOAN HEMOGLOBIN.

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We isolated a cDNA encoding assimilatory nitrate reductase (NR) of the marine Raphidophyte *Heterosigma akashiwo* from a library constructed in lambda ZAP Express page. Clones were screened

with a monoclonal antibody NR10 raised against NR from the marine red alga *Porphyra yezoensis*, which has the cross reactivity to molybdopterin domain. The 2.9 kb clone contained an open reading frame of 2,797 nucleotides that hybridized to a 2.7 kb mRNA. The deduced amino acid sequence contained 931 residues, with a predicted molecular weight of 103,633. Amino acid sequence comparison with other assimilatory NRs has shown that NR from *H. akashiwo*, as well as typical eukaryotic assimilatory NRs, includes three functional domains, each of which contains molybdopterin, heme, and FAD as prosthetic groups. However, the heme and the FAD domains are separated by a polypeptide of 116 amino acids, which has 35 to 42% identity to hemoglobins from the cyanobacterium *Nostoc commune*, the green alga *Chlamydomonas eugametos*, the ciliated protozoa *Paramecium caudatum*, and *Tetrahymena pyriformis*. The protozoan hemoglobins comprise distinct group from the well-known hemoglobin and myoglobin family that includes leghemoglobin as well. This finding not only provides new insight into evolution of eukaryotic NRs but also suggests the existence of various eukaryotic NRs differing in domain composition.

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#### ULTRASTRUCTURAL CHANGES AND GROWTH RESPONSE OF *EUGLENA GRACILIS* TREATED WITH INORGANIC AND ORGANIC TIN COMPOUNDS

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The toxicity of heavy metals have been studied in a variety of organisms, including algae but the effects of organometallic compounds have received much less attention. Organotin (OT) compounds are extremely poisonous and have been widely used as active ingredients in antifouling paints, as PVC stabilizers, industrial and agricultural biocides. Its extreme toxicity has resulted in adverse biological effects on non-target organisms. In the present study, the effect of inorganic tin (SnCl<sub>4</sub>) and organotin compounds (TeBT and TBT) on *Euglena gracilis* was evaluated. The toxicants were added in series ranging from 0 to 50 µg ml<sup>-1</sup>. Growth inhibition was observed in the OT-treated cultures, being most severe the effect of TBT. Ultrastructural damage was analyzed by means of TEM. Recently, we had reported the lethal effect of 50 µgml<sup>-1</sup> of TBT on *E. gracilis*: no organelles could be distinguished after 96 hs exposure. To determine the sequence of alterations prior to the cell death, sublethal concentrations (0 to

2000 µg L<sup>-1</sup>) of TBT were also assayed. Functional and structural alteration of mitochondria might be the cause of the remarkable accumulation of paramylon in giants cells. Chloroplasts showed a severe disorganization and swollen membranes. The Golgi bodies cisternae appeared dilate filled with conspicuous secretion material. It may be concluded that the organometallic compounds would be more toxic than the inorganic salts, suggesting that they are more efficiently absorbed for its lipid solubility, and/or less excreted because of the strength of the cysteine (protein)-OT bond.

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#### NITRATE REDUCTASE ACTIVITY IN GRACILARIACEAE (GRACILARIALES, RHODOPHYTA).

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Most of the agar produced today is obtained from Gracilariaceae cultivated in ponds or directly in the sea. Therefore, there is a great interest in understanding the mechanisms of nutrition and growth of those algae. Nitrogen is the major nutrient limiting macroalgal production and its major source in the ocean is in the form of nitrate, which must be reduced to integrate the nitrogen-containing biomolecules. Nitrate reductase (NR) is the crucial first enzyme in the nitrogen reduction pathway and is the rate-controlling step which regulates the input of nitrogen into the organisms. In this paper we compare some of the biochemical features of the NR activity from three commercially cultivated species, *Gracilaria tenuistipitata*, *Gracilaria chilensis* and *Gracilariopsis tenuifrons*. Some of the factors regulating NR activity, optimal pH (8.0) and circadian oscillation were found to be similar for the three species, but other factors varied. For *G. tenuifrons* optimal temperature was 30 °C, optimal NADH concentration was 0.01 mM, the activity plateau was reached after 60 min, and the enzyme was quite stable in the crude extract when kept at 4 °C (90% of activity remains after 48 h). For *G. chilensis* optimal temperature was 20 °C, optimal NADH concentration was 0.04 µM, the activity plateau was reached after 10 min, and the enzyme was less stable in the crude extract when kept at 4 °C (17% of activity after 48 h). These results are compared to previous results obtained for *G. tenuistipitata*.

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**FEEDING INTERACTIONS BETWEEN NATIVE OHIO RIVER MUSSELS (BIVALVIA: UNIONIDAE) AND ZEBRA MUSSELS (*DREISSENA POLYMORPHA*).**

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We evaluated effects of zebra mussel infestation on the feeding of native unionids in the Ohio River through gut content analysis. In 1996, mean algal cell numbers in guts of heavily infested and lightly infested *Amblema plicata* ( $5.7 \times 10^5$  versus  $9.1 \times 10^5$ , respectively) were not significantly different ( $p = 0.17$ ). But, heavily infested *A. plicata* had lower ( $p < 0.01$ ) mean ash-free dry weight (AFDW) in guts (1.4 mg) than lightly infested ones (4.6 mg). Mean algal cell number and AFDW from heavily infested *Quadrula pustulosa* ( $1.8 \times 10^4$  and 0.6 mg AFDW) also were lower ( $p \leq 0.05$ ) than that of lightly infested ones ( $3.9 \times 10^5$  and 1.8 mg AFDW, respectively). In 1997, significant reductions ( $p \leq 0.05$ ) in total algal cells and AFDW in guts also occurred for heavily vs. lightly infested *A. plicata* and *Q. pustulosa*. The guts of all zebra mussels attached to individual unionids from Ohio River Mile 397 contained 50% more ( $p \leq 0.05$ ) cells ( $2.3 \times 10^5$  versus  $9.4 \times 10^4$ , respectively) and AFDW (4.55 mg versus 0.92 mg) than individual infested unionids. Algal taxa at the sediment-water interface were essentially the same as those in guts of all mussels, confirming significant diet overlap. Thus, algal and detrital food resources of unionid guts are greatly reduced by feeding of zebra mussels. Reductions in unionid ingestion is likely a primary mechanism for reduced glycogen levels previously reported for heavily-infested unionids.

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**PSEUDO-NITZSCHIA IN LOUISIANA COASTAL WATERS: MOLECULAR PROBE FIELD TRIALS**

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Weekly (estuarine) and monthly (shelf waters) examination of the phytoplankton population in 1997

revealed the presence of at least six species of *Pseudo-nitzschia* (*P. pungens* (Grunow) Hasle, *P. multiseriis* (Hasle) Hasle, *P. pseudodelicatissima* (Hasle) Hasle, *P. delicatissima* (P. T. Cleve) Heiden, *P. subfraudulenta* (Hasle) Hasle, and *P. sp. cf. americana*). Domoic acid was detected in net tow samples indicating active toxin production. Spring and fall blooms occurred at much lower levels than in the previous seven years and consisted of a mixture of species. *Pseudo-nitzschia multiseriis* dominated at the estuarine site during late winter, whereas *P. pungens* exhibited a summer dominance in shelf waters. In addition to differential interference contrast (DIC) and scanning electron microscopy (SEM), the whole cell hybridization technique, utilizing species-specific large-subunit ribosomal RNA-targeted oligonucleotide probes, was field tested for its ability to aid in detection and quantifying the four most common species occurring in Louisiana coastal waters (first four listed above). Probes successfully detected their presence, even at levels as low as 0.1% of the total phytoplankton population. Probe estimates of the concentrations of the four species, however, differed from DIC/SEM results. This discrepancy appears to stem from the presence of unlabeled cells due to poor physiological state or species variability. Alternatively, the difference could reflect inherent differences between the two counting methods, as the probes detect live planktonic cells, whereas DIC/SEM count all cells: live, dead, and hidden in fecal pellets and consumers. Nevertheless, these results demonstrate the promise of probes in shellfish monitoring programs.

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**FACTORS REGULATING ACCUMULATION OF CRYPTOPHYCIN-1 IN THE CYANOBACTERIUM *NOSTOC SP. GSV 224***

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The cryptophycins are cytotoxic depsipeptides produced by cyanobacteria of the genus *Nostoc*. Extensive *in vivo* studies on cryptophycin-1 and a broad range of natural, synthetic and semi-synthetic analogs has demonstrated potent antitumor activity against a wide range of murine and human solid tumors. One such analog is currently being evaluated in Phase I human clinical trials. To assess the potential for commercial production of cryptophycin-1 (the most abundant naturally occurring congener), we have initiated a program aimed at identification of the biological and physicochemical factors that influence growth and cryptophycin

accumulation in axenic laboratory cultures. The effects of concentration and chemical form of major and minor nutrients, including nitrogen, phosphorus, magnesium, calcium, and iron, have been established by determining the cryptophycin-1 content of control and treated cultures using a sensitive HPLC assay. Cryptophycin-1 is produced by actively growing cultures and accumulates in both the cell mass and in the surrounding growth medium. Unlike most cyanobacterial secondary metabolites examined to date, the accumulation of cryptophycin-1 in culture is profoundly dependent upon incubation conditions, exhibiting relatively narrow pH and nutrient concentration optima that are not coincident with optimal conditions for biomass accumulation.

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#### **MODELING THE DAILY AND ANNUAL PRODUCTION OF EPIPHYTIC MACROALGAE GROWING ON MANGROVE ROOTS**

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A production simulation model was developed to quantify daily and annual primary production of two dominant species of epiphytic macroalgae, *Bostrychia calliptera* (Montagne) Montagne, and *Caloglossa leprieurii* (Montagne) J. Agardh growing on mangrove roots in a tropical estuary, Pacific Coast of Colombia. The model used hourly measurements of solar radiation, and irradiance below the mangrove canopy, as forcing functions. The model which was validated using hourly measurements of photosynthesis and of monthly biomass production, showed good agreement between observed and predicted values ( $r^2 = 0.72$ ,  $p < 0.01$ , and  $r^2 = 0.86$ ,  $p < 0.01$  respectively). The sensitivity analysis revealed incident irradiance, and attenuation by the overlying water as the main factors that generally resulted in lower estimates in the daily production rates. Algal production was significantly higher ( $p < 0.01$ ) during dry season (February to June) than during the rainy season (October-December). Significant differences in daily production were found to correlate with the number of hours they are exposed, and the time at which low tide occurred during the day ( $p < 0.01$ ). Thus, if low tide occurred at solar noon (high irradiance) production rates were lower than when low tide occurred in early morning or late afternoon (low irradiance). This is consistent with the P-I curves where the algae demonstrated an adaptation to shade. The annual net production of the two species of macroalgae was estimated at  $469 \text{ g C m}^{-2} \text{ yr}^{-1}$ , which is equivalent to

about 9% of the annual leaf carbon production of the mangal.

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#### **DIVERSITY OF COCCOID ALGAE ISOLATED FROM THE ARROWWOOD NATIONAL WILDLIFE REFUGE LAKES, NORTH DAKOTA, DURING WINTER**

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Unicellular asexual coccoid green algae (Chlorophyta) are ubiquitous in freshwater ecosystems. Little is known about the distribution and diversity of these organisms, primarily because of their simple morphology and small size. In addition, other green-colored coccoid algae, such as xanthophytes and eustigmatophytes, may confound identification. In this study, we examined the diversity of asexual coccoid algae in the shallow lakes of Arrowwood National Wildlife Refuge (ANWR) near Pingree, North Dakota. For 3 consecutive winters (1994 to 1997), phytoplankton samples were collected at 4 sites on 3 lakes in the ANWR and a collection of 90 isolates of coccoid algae was developed. A hierarchical approach was used to examine the diversity of these isolates. The methods in this approach included HPLC analysis of photosynthetic pigments, taxon-specific PCR primers, and PCR-RFLP of the 18S rDNA. Thus far, six 18S rDNA genotypes of coccoid trebouxiophytes, 3 genotypes of coccoid chlorophytes, and 1 genotype of coccoid eustigmatophyte have been detected from these isolates. These results suggest a higher level of diversity of coccoid algae than has been reported in regional floras.

### 112

#### **MOLECULAR INSIGHTS INTO THE EVOLUTIONARY HISTORY AND BIOGEOGRAPHY OF HAWAIIAN SPECIES OF SARGASSUM**

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The ecologically important Pan-Pacific genus *Sargassum* was used to examine, for the first time, evolutionary and speciation processes in Hawaiian marine plants. *Sargassum* makes an excellent candidate for this type of research; in the Pacific it has over 300 hundred species, offering an unparalleled opportunity for phylogenetic and biogeographic reconstruction. Within the Hawaiian

archipelago the 5 endemic species of *Sargassum* are wide-spread and well defined ecological dominants. Molecular sequencing of a rapidly evolving chloroplast gene region (rbcL-IGS-rbcS) was used to examine introduction and speciation events. Over 35 sequences representing all four subgenera and half the family Sargassaceae were used to construct a phylogeny. At the genus level, this modern treatment confirms much of the traditional taxonomic treatment but is in sharp contrast to traditional evolutionary concepts within the genus. In terms of evolutionary history, rbcLS spacer data suggest that at least two introductions have given rise to Hawaiian *Sargassum* species. A Californian link is suggested for two of the species, representing the first empirically based indication of biogeographic affinities for Hawaiian macro-algae. Molecular data also suggest that speciation is occurring within this genus in an east to west direction in support of the vortex biogeographic model. These data refute J. Agardh's evolutionary concept within the genus, provide insight into the evolutionary history and biogeography of Hawaiian marine algae and lay an important foundation for future research.

### 113

#### **CHEMOTAXONOMIC APPROACHES FOR ASSESSING ENVIRONMENTAL CONTROLS AND BLOOM POTENTIALS OF NATURAL PHYTOPLANKTON COMMUNITIES**

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Phytoplankton communities are composed of diverse microalgal species, each with unique physiological requirements for optimal growth. Species composition is regulated by the synergy of environmental and ecological conditions. A process-based understanding of the factors responsible for noxious/harmful algal blooms is necessary for developing predictive models and designing effective management strategies for mitigating the negative impacts of persistent blooms. Microalgal photosynthetic pigments (chlorophylls & carotenoids) are useful biomarkers for quantifying the relative abundance of algal groups (dinoflagellates, cyanobacteria, diatoms, etc.) in mixed assemblages. Photopigment radiolabeling techniques can provide measurements of the carbon-specific growth rates of algal groups. Algal growth rates are sensitive indicators of physiological responses to alterations in environmental conditions and provide a rapid means of determining algal bloom potentials. The Neuse River

Estuary, NC is experiencing an increase in the frequency, areal coverage, and duration of nuisance algal blooms. Chemotaxonomic approaches were coupled with mesocosm (55 liter) and cubitainer (4 liter) bioassays to determine Neuse River phytoplankton community responses to nutrient (ammonium, nitrate, phosphate) additions and mixing. Experimental results suggest that phytoplankton productivity is N-limited. Mixed conditions promote diatom growth while phytoflagellate abundance is enhanced under static (non-mixed) conditions. The community exhibited differential responses depending on the N source (nitrate vs. ammonium). Ammonium additions promoted higher cyanobacterial growth and biomass accumulation than nitrate amended treatments. Increasing anthropogenic ammonium inputs through atmospheric deposition may support potentially harmful cyanobacterial blooms in estuarine and coastal waters.

### 114

#### **MOLECULAR ANALYSIS OF BACTERIA IMPLICATED IN SYNTHESIS OF SAXITOXIN**

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It is becoming increasingly obvious that bacteria play a role in the synthesis of certain phycotoxins. Several bacterial strains isolated from cultures of toxigenic dinoflagellates have been shown to be capable of autonomous production of compounds that are thus far indistinguishable from saxitoxins. These bacteria contain genes that direct the synthesis of saxitoxin-like compounds and these genes are potentially more tractable targets for isolation and manipulation than are the putatively homologous dinoflagellate genes. To identify these genes, we have pursued a molecular genetic approach with six bacterial strains and found that each was resistant to most antibiotics routinely used for selection of transposon-derived mutants and that four strains had high nuclease activity that further hindered attempts to produce mutants. Nonetheless, a strain of *Pseudomonas stutzeri* associated with a toxic *Alexandrium lusitanicum* could be mutagenized and transconjugants selected on either Km or Gm if cells were grown in a freshwater (rather than sea water) medium. We subsequently selected mutants that were sensitive to Gm in sea water and found that a Tn5 element was inserted into a two-component histidine kinase gene that is specifically responsive to

calcium/ammonium/magnesium. We have also selected ARG mutants because saxitoxins are built from three arginine moieties. Eight mutants have been found that are unable to transport and/or utilize exogenous arginine supplied as the sole carbon/energy/nitrogen source. We have just begun to screen for mutants unable to synthesize saxitoxin and hence, are expected to have Tn elements inserted into the genes required for saxitoxin synthesis.

### 115

#### **DIATOM PROTOPLASTS ARE AMOEBOID DURING RECOVERY FROM OSMOTIC SHOCK**

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Cells of the diatom *Ditylum brightwellii* were briefly plasmolysed in 0.2-0.4M NaCl and their recovery in sea water was followed by time-lapse video microscopy. Spherical protoplasts shrank to about an eighth of the cell volume and were free within the valve, rotating and moving gently. After about 15-20 mins., numerous active filopodia began to extend randomly from the protoplasts. The tips of these ran over the inside of the valve and soon appeared to attach to specific sites around the end of the valve. The filopodia appeared tensile, often pulling the protoplast to one end of the empty wall before connection was established to the other end, whereupon it became central. Most cells then slowly expanded and appeared completely normal within about 1.5 hrs. Furthermore, several cells were recorded dividing soon afterwards, so we presume recovery is complete. If cells had been dividing at plasmolysis, they shed their forming valves. Upon recovery, if the valves were small, the filopodia did not "recognize" them, growing past them and later, when the two protoplasts inflated, they pushed the nascent valves aside. Some of these cells initiated completely new valves, apparently therefore being able to restart valve morphogenesis. If the shed wall was larger, however, filopodia attached to its edge, and the daughter cells soon expanded and looked normal. These observations show the remarkable resilience to the cells toward osmotic shock. They also indicate that the mature valve has specific anchoring points inside it which the protoplast recognizes and specifically attaches to. These anchor points are likely to be significant in the spatial control of cell division and morphogenesis.

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#### **PHYSIOLOGICAL EFFECTS OF NITRATE LIMITATION ON THE TOXIC DINOFLAGELLATE ALEXANDRIUM TAMARENSE FROM CASCO BAY, MAINE**

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At least one strain of the harmful algal bloom genus, *Alexandrium*, can take up nutrients, specifically nitrate, in the dark during nocturnal descent of diel vertical migration (DVM). There is, however, widespread variability among strains of *Alexandrium*, as shown in studies of morphology and toxin composition of isolates from the Gulf of Maine. Our objective is to study the effects of nitrate limitation on a Casco Bay strain of *Alexandrium*, focusing on behavior and nutrition as indicators of nutrient status in the field. The effect of nitrate availability on an isolate of *Alexandrium tamarense* from Casco Bay, Maine was examined for physiological and behavioral changes in a 2-m, thermally stratified laboratory water column (tank). Temperature ranged from 17 °C at the surface to 7 °C at 200 cm with a surface irradiance of 400  $\mu\text{mol quanta m}^{-2}\text{s}^{-1}$  (14h light:10h dark cycle), identical to a previous study using a Gulf of St. Lawrence isolate. Like the St. Lawrence strain, the cells maintained a thin surface layer, depleting nitrate (initially 50  $\mu\text{M}$ ) from the top 20 cm within a few days. Unlike the St. Lawrence strain, which acquired nitrate through nocturnal migration to the nitracline, the Casco Bay cells did not deplete the tank of nitrate and showed signs of progressive N limitation. Calculations show that nocturnal nitrate uptake was inadequate to sustain a growth rate of 0.3  $\text{day}^{-1}$  as determined from nitrate replete cultures. It is concluded that the Casco Bay isolate is incapable of obtaining and sustaining growth from nocturnal descent to the nitracline.

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#### **THE DIATOM GENUS TRYBLIOPTYCHUS HENDEY: FINE STRUCTURE AND SYSTEMATICS**

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*Tryblioptychus* Hendey is a diatom genus established to accommodate a small elliptical species with alternating hyaline and areolate sectors on the valve face originally

described by Cleve as *Campylodiscus cocconeiformis*. Until now, the systematic position of this genus was unclear with various authors placing it in Surirellaceae, Actinodiscaceae, and Thalassiosiraceae, families with little taxonomic affinity. An abundance of material identified as *Tyrblioptychus cocconeiformis* from the F. Meister collection provided an opportunity to study the fine structure of this diatom and, thus, to clarify its taxonomic relationships. The presence of loculate areolae with external foramina and internal dome-shaped cribra, a marginal ring of fulcrotubulae with three struts and a subcentral fulcrotubula with four struts, a single marginal rimoportula with strong external development, and a cingulum composed of perforated bands justify Simonsen's placement of this genus in the Thalassiosiraceae. The affinities of a second species in this genus, *T. hainansis* Voigt, remain undetermined. The presence of *T. cocconeiformis* in samples from coastal waters near China, Japan, Vietnam, Singapore, Sumatra, India and the West Africa indicate that the species is widely distributed in warm waters.

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**LIGHT AND SCANNING ELECTRON MICROSCOPE OBSERVATIONS OF AN INTERESTING SYNEDROID DIATOM FROM FLORIDA BAY, USA**

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Light microscopic examination of water and net samples from several sites in Florida Bay revealed the presence of large numbers of spindle-shaped synedroid diatoms bearing a superficial resemblance to other spindle-shaped diatoms, especially *Nitzschia longissima* (Breb.) Ralfs, *Cylindrotheca closterium* (Ehrenb.) Reimann et al., *Toxarium hennedyanum* (Greg.) Pelletou, *Psammosynedra closterioides* (Grun.) Round, *Synedropsis hyperborea* (Grun.) Hasle et al. Scanning electron microscope observations of this diatom revealed the presence of a single row of small poroid areolae at the valve margin, a single rimoportula near each pole, and 2 to 3 tiers of simple pores on the polae mantle. This combination of features does not match any of the existing generic concepts.

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**TIME SCALES OF PHOTOSYNTHETIC RESPONSE TO ULTRAVIOLET RADIATION AND TEMPERATURE BY SUBARCTIC PHYTOPLANKTON**

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A series of assays were conducted on the phytoplankton of a subarctic lake to determine whether short-term (hours) temperature and ultraviolet radiation (UVR) effects on photosynthesis were indicative of photosynthetic and growth responses at longer time scales (days). A series of short-term experiments (2-4 h incubations) showed that photoinhibition under strong PAR was 100-350 % greater in the presence of UVR. A temperature x UVR interaction was evident in one experiment with greater net damage at 10 °C, but the lack of interaction in two other experiments indicated that the time scale examined was too short for the temperature-dependence of repair processes to be detected. A series of long-term experiments (8 d) showed that over time the photosynthesis-irradiance (P-E) parameters deviated substantially from those obtained initially. By Day 7,  $\alpha$  and  $P_{max}$  were 67 and 30% higher in the -UVR 10 °C treatment relative to +UVR 10 °C, but there was no evidence of increased resistance to photoinhibition, as measured by the  $b$  parameter. Contrary to the UVR effect on photosynthesis, growth rates as estimated by changes in chlorophyll *a* were not significantly different between UVR treatments after 8 d. We conclude that acute and chronic responses of photosynthesis to UVR are not the same, and that short-term photosynthetic responses provide a poor guide to longer term growth effects. Temperature plays a role in the observed UVR response at both time scales.

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**SYSTEMATICS AND BIOGEOGRAPHY OF THE COMPSOPOGONALES (RHODOPHYTA) WITH EMPHASIS ON FRESHWATER GENERA IN NORTH AMERICA**

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The rhodophyte order Compsopogonales consists of three families: Compsopogonaceae, Boldiaceae and Erythropeltidaceae. The first two families are freshwater in their occurrence while the members of the third family are marine. The validity of this order as a natural monophyletic group was examined using DNA sequence data from both the chloroplast (*rbcL*) and nuclear (18S rRNA) genome for representative genera of these families and related orders. Both parsimony and distance analyses weakly support a monophyletic

compsopogonalean clade excluding the genus *Smithora*. A close relationship between the two freshwater families is strongly supported. In comparisons of both genes of the compsopogonacean genera, *Compsopogon* and *Compsopogonopsis*, sequence divergence was very low (0 to 0.66% rbcL in 5 collections and 0 to 0.12% 18S in 2 collections). This result suggests that these genera should be synonymized. The level of genetic divergence in North America for genera in the freshwater families (10 collections of Compsopogonaceae and 9 collections for the Boldiaceae) was assessed using DNA sequence analysis of rDNA internal transcribed spacer regions (ITS1 and ITS2). Biogeographic patterns will be discussed.

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#### **PRELIMINARY OBSERVATIONS ON AGE AND GROWTH IN *LITHOTHAMNION CRASSIUSCULUM*, A RHODOLITH-FORMING SPECIES IN THE GULF OF CALIFORNIA**

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The nongeniculate coralline alga *Lithothamnion crassiusculum* is commonly found as a free-living rhodolith in the wave-exposed shallow waters of the southwestern Gulf of California. This species has bands along the main branch axes suggesting growth is periodic and the bands might be used to determine age and growth. As a part of our ongoing research related to rhodoliths and their ecology, we have made preliminary observations on the nature of these bands based on their structure in entire plants and on field growth experiments. Individual plants from different size classes were tagged, stained with Alizarin and returned to the field. Half of the plants were sampled after six months and the rest after twelve months. The data indicate that this species grew at a rate of 0.67mm per year. There were significant differences among branches within a plant, but not among plants of different size. Growth was seasonal, with high rates in winter-spring and lower rates in summer-fall. This may explain the observed alternating light-dark bands along branch axes. The growth rates suggest that larger plants (ca. 15 cm dia.) may be over 100 years old.

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#### **HISTONE-LIKE PROTEINS FROM ISOLATED NUCLEI OF THE MARINE DINOFLAGELLATE *GONYAULAX***

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We have developed a method for the isolation of intact nuclei from *Gonyaulax polyedra* and *G. tamarensis*, to permit characterization of the nuclear basic proteins. The combination of a very tough theca, and extremely fragile nuclear envelope provided the major obstacles in this endeavour. However, the availability of intact nuclei from *Gonyaulax* will permit many other studies in addition to the characterization of histone-like proteins reported here. The isolation procedure included cell harvest by filtration through Nitex, cell disruption by sonication, and nuclear purification by low-speed centrifugation and filtration through Nitex. Unlike most dinoflagellates, *Gonyaulax* nuclei are "C-shaped" rather than spherical in morphology, but are similar to other dinoflagellate nuclei in staining properties with various dyes, and the presence of permanently condensed chromosomes. As in other dinoflagellates, *Gonyaulax* nuclei are devoid of histones, but the chromatin does contain histone-like proteins. Thus basic proteins prepared from chromatin of isolated nuclei of both *G. polyedra* and *G. tamarensis* appear as one major band of about 13,000 daltons in SDS gels. In Western blots, these histone-like proteins cross-react with antibodies prepared against histone-like proteins from the nonphotosynthetic heterotrophic dinoflagellate *Cryptocodinium cohnii*. We conclude that histone-like proteins in dinoflagellates are conserved since they are similar in a non-photosynthetic heterotrophic dinoflagellate and a typical phototroph.

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#### **THE ULTRASTRUCTURE OF *GYMNODINIUM SANGUINEUM*: FLAGELLAR APPARATUS AND CYTOSKELETON**

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The large dinoflagellate *Gymnodinium sanguineum* has been studied extensively with serial sectioning and transmission electron microscopy. The flagellar apparatus consists of basal bodies that are nearly anti-parallel, a broad microtubular root, a striated connective between the microtubular root and the long transverse striated fiber, diminutive striated collars around each flagellar exit aperture, and a broad striated ventral connective that connects to the cell's left ventral surface of the sulcus and to the microtubular root. A lamellar structure with distinct fibrillar extensions, similar in

appearance to those of a multi-layered structure (mls), has been observed within the flagellar apparatus. A large ventral ridge is present and no nuclear connective has been observed. Cytoskeletal details previously observed with fluorescence microscopy have been detailed ultrastructurally and will also be presented.

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#### **APPLICATION OF SINGLE CELL GEL ELECTROPHORESIS (COMET ASSAY) TO DETECTION OF DNA DAMAGE IN PROTISTS**

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Single cell gel electrophoresis (SCGE) under denaturing alkaline conditions has proven useful for detecting single-strand DNA breakage in nuclear genomes of individual eukaryotic cells. This method, dubbed "comet assay" for the cometary form of observed cellular DNA migration, allows visualization and may allow quantification of DNA damage induced in individual nuclei by diverse agents including oxidants, ionizing and -- indirectly through nucleotide excision repair-associated single-strand breakage - ultraviolet radiation. Adapting published methods for mammalian cells, we have used SCGE to assay UV-induced DNA damage in a eukaryotic freshwater alga, *Poterochromonas malhemensis*, extending the reported application of this technique beyond animals and plants. The potential utility of SCGE in studying DNA damage in diverse photosynthetic eukaryotes will be discussed.

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#### **STUDIES OF THE INTERACTIONS BETWEEN EUGLENA GRACILIS AND DIFFERENT STRAINS OF PATHOGEN MICROORGANISMS**

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Studies made in polluted water streams showed that one of the species found was *Euglena gracilis* (Euglenophyta) sharing the habitat with pathogen bacteria. Our study was aimed to analyze if there was any interaction between *E. gracilis* and strains of bacteria isolated from a stream and human pathologies. To evaluate this relation, different assays were carried out. Cultures of *E. gracilis* growing with *Klebsiella* sp. and other growing with *Escherichia coli* were made. After 48 h., in cultures of *E. gracilis-Klebsiella* sp., the algae changed its shape, turned oval, lost movility and

presented abundant paramylon grains. In cultures of *E. gracilis-E. coli*, the algae presented a normal shape but abundant paramylon. The greatest algal growth was registered when growing with *E. coli*. On the other hand, we used antibiograms, where different strains were exposed to an etilic ether extracts of *E. gracilis*. The strains used were *Pseudomonas aeruginosa*, *Salmonella* sp., *Klebsiella* sp., *Escherichia*, *Mycobacterium fortuitum*, *Mycobacterium gordoneae* and *Staphylococcus aureus*. As a result of these experiences, positive interaction was observed with *Klebsiella* sp. (inhibition), *M. fortuitum* and *M. gordoneae* - with these two an induction ring appeared first and then and inhibition ones. No answers were detected with the other strains used with this technique. We conclude that different interactions can be observed depending on the methodology employed. The next step is to analyze the substances involved in these relations and the conditions that determine their liberation.

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#### **DESIGN STRATEGIES FOR FORECASTING SYSTEMS FOR HARMFUL ALGAL BLOOMS**

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A long term objective of the ECOHAB program is to develop the capacity to forecast harmful algal blooms. Our research efforts have focused on the development of synoptic sampling approaches which can be assimilated into numerical forecasting models and/or be used to empirically predict the presence or absence of an algal taxon. One approach we have been exploring is the use of artificial neural networks which unlike traditional empirical approaches, are effective at describing complex nonlinear phenomena. This is particularly important when environmental data is used to predict the presence of a specific species in a heterogeneous algal community. The strengths and weakness of neural networks in predicting phytoplankton community composition will be demonstrated using several large environmental databases. A second forecasting approach is based on data assimilation into numerical models. This requires input data and appropriate physical/biological models. Optical data can provide synoptic data over ecologically relevant scales; however the data acquired provides only bulk signals for the

optically active constituents within the water. Recent optical deconvolution techniques show much promise and species-specific algorithms have been developed to predict the presence or absence of the toxic red-tide dinoflagellate, *Gymnodinium breve*. The species-deconvolution technique utilizes fourth derivative spectra and a similarity indexes based upon a reference *G. breve* absorption spectra and the measured phytoplankton community absorption spectra. Data from natural populations in the Gulf of Mexico will be used to demonstrate the merits of this approach. The resulting optical data is amenable for data assimilation into numerical forecasting strategies. The design and operation of one such data assimilation system developed for the coastal waters of New Jersey will be discussed.

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**APPLICATION OF DNA PROBES FOR DETECTION OF HARMFUL ALGAL SPECIES IN CULTURED AND NATURAL SAMPLES**

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A common problem associated with studies of harmful algal blooms (HABs) is identifying a particular causative organism as it occurs in nature, especially when rapid analysis of a large number of samples is required. Development and application of species-specific molecular probes has long been heralded as the means by which one might accomplish this goal with greater ease and efficiency than might be possible using traditional morphotaxonomic-based techniques. Over the past decade considerable effort has been spent developing novel probes for a variety of HAB organisms and testing their sensitivity and specificity, largely in the context of cultured samples. In contrast, comparatively little work has been done to identify the needs of the likely end-user of these probes, to gauge the specificity of the probes in the context of natural samples, and to reveal the costs and benefits of different probe application techniques as well as their inherent limitations when one considers realistic scenarios of how they might be applied routinely. To address these issues using *Pseudo-nitzschia* and *Alexandrium* as model HAB species, existing ribosomal RNA (rRNA) targeted probes were distributed to a number of researchers studying those organisms in several different labs within the US and abroad. This multi-year effort, known as TACO (Toxic Algae Cook Outs), has revealed numerous insights as to the utility of the probes and helped identify certain problems associated with their

use. In turn, this information has been used to refine probe application techniques and highlight needs for future research.

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**IS THE "SPORE WALL" OF CORALLINE RED ALGAE A "CALCIUM DEPOSITION MATRIX"?**

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Transmission electron microscopy has shown that prior to release, mature spores of all investigated red algae are coated by a presumptive mucilage layer within the sporangial cell wall. Although terminological differences exist concerning the various wall layers of reproductive cells, to our knowledge a true "spore wall" to the inside of the mucilage coat has not been demonstrated except in tetrasporangia of the coralline alga *Halimnion* studied by Vesik and Borowitzka in 1981. Investigations in our lab of later stages of sporogenesis in approximately 20 genera in six of eight corallinacean subfamilies indicate that a conspicuous wall layer, whose morphology varies somewhat across genera, is formed adjacent to the cell membrane in bi-/tetrasporangia and carposporangia. Since it is known that calcification in coralline algae is enhanced by photosynthesis and requires an organic wall matrix wherein calcite crystals are deposited, it is our supposition that the unique "spore wall" of coralline algae represents a "calcium deposition matrix" which can provide the attached spore and young germlings an early start in thallus calcification. Further studies are underway to precisely determine when calcification first occurs in the transition from mature spore to young germling.

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**THE RATIO BETWEEN ECOLOGICALLY-SIMILAR GAMETOPHYTES AND TETRASPOROPHYTES IN POPULATIONS OF THE GIGARTINACEAE: DEMOGRAPHIC MODELS**

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In populations of the Gigartinaceae, gametophytes often predominate numerically over tetrasporophytes on an

annual and/or a whole-habitat basis. A number of hypotheses have been proposed to explain this pattern, based on the usually-implicit assumption that the equilibrium gametophyte:tetrasporophyte (G:T) ratio should be 1 when both reproductive phases are ecologically similar. We present demographic models that show that the equilibrium G:T ratio differs from 1 when both phases are ecologically similar. Model assumptions are that there are no differences in vital parameters (except spore output) between phases. The relative spore output represents the simplest possible case: 1 carpospore is produced every two (female-male) gametophytes (considering dioecious species), which originates 1 tetrasporophyte, and 4 tetraspores are produced by every tetrasporophyte, which originate 2 female and 2 male gametophytes. Under different combinations of mortality rates (the same for both phases for each model) and initial G:T ratios, the G:T ratio generally stabilizes around 2.83 after a variable number of iterations of the models. We conclude that the idea of an "expected" G:T ratio equal to 1 for ecologically-similar phases should be abandoned. The G:T ratios observed in nature would derive from our simplest possible scenario by differential variations between phases of spore output, spore viability, sporeling recruitment, reproductive maturation rates, reproductive periodicity, reproductive life-span, competitive abilities, resistance to herbivores and pathogens, and adult mortality rates, and by possible apogamy and apomeiosis.

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**THE COMPARATIVE ECOLOGY OF ALGAL SPECIES: A CASE STUDY OF THE GIGARTINACEAE (RHODOPHYTA)**

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Understanding population differentiation requires combining studies from ecology, phylogeny, taxonomy and gene flow. The objectives of this paper are to summarize the ecology of species in the Gigartinaceae and, secondly, to point out what kinds of ecological studies are still needed and how they might be completed. Field studies in this family can be hampered by an inability to distinguish between morphologically similar species. An examination of *Mazzaella splendens* and *M. linearis* showed that early developmental stages of the annual uprights provide useful vegetative characters, and a common garden experiment indicated that character differences were not due to plasticity. Factors affecting the distribution of uprights are

important in determining whether a species can maintain space and reach reproductive maturity. Water motion and interspecific competition limit the distribution of uprights for some species in this family; endophytes, nutrient availability, and desiccation may also be significant. Species distributions may be set by how some of these extrinsic factors act on dispersal or on recently settled spores. Biomechanical studies showed that fragmented blades from a low wave impact species could survive at a high wave impact site which implied that it does not disperse to or cannot attach at high impact sites. Using a comparative ecological approach allows conclusions to be made about how algal species survive in their respective habitats. If this strategy was combined with studies of gene flow in the Gigartinaceae, our understanding of how genetic variation is maintained in algal populations would also be enhanced.

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**A PROPOSAL FOR A NEW RED ALGAL ORDER, THE BALBIANIALES**

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The freshwater rhodophyte *Balbiania investiens* was collected as an epiphyte of *Batrachospermum helminthosum* in a small stream in Ambelside, England. Unialgal cultures and field collections of *Balbiania* were analysed in terms of their morphology, ultrastructure and DNA sequences of the *rbcL* and 18S rRNA genes. The major distinguishing feature of this genus is the production of spermatangia on the tips of specialized, elongate cells. The spermatangia are cut off in a cluster together with non-spermatangial cells that are packed with starch granules. The only other rhodophyte genus with this feature is *Rhododraparnaldia*; the two genera are differentiated from each other by the pattern of branching. In the parsimony and distance trees of the two genes analyzed, *Balbiania* and *Rhododraparnaldia* are grouped together on a branch separate from other taxa of the Acrochaetales, Batrachospermales, Nemaliales and Palmariales. This branch is well supported by bootstrap resampling. Based on these findings, we propose the establishment of a new order, the Balbianiales, which includes *Balbiania* and *Rhododraparnaldia*.

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### A COMPARISON OF FRESHWATER AND MARINE *HILDENBRANDIA* (RHODOPHYTA) IN NORTH AMERICA

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The phylogenetic relationships between marine and freshwater *Hildenbrandia* (Rhodophyta) are not well understood. Freshwater *Hildenbrandia* collections may represent invasions by marine populations into stream habitats. To test this hypothesis we have employed several comparative techniques to examine 15 collections of marine and freshwater *Hildenbrandia* from Newfoundland to Costa Rica. Morphometric analysis of 10 vegetative and reproductive characters was performed using cluster analysis (UPGMA algorithm), principle components analysis and one-way analysis of variance. DNA sequence comparisons of the *rbcl* and 18S rRNA genes were accomplished using parsimony and distance analyses. Pit plug morphology was studied using transmission electron microscopy. Morphometrically, the groups of populations are separable based on thallus and filament height, as well as number of cells per filament. A wide range of DNA sequence divergence values was found for the *rbcl* gene, ranging from 0.2% to 26.9%. Both the molecular phylogenies and the morphometric phenograms show distinct groupings of marine and freshwater populations. However, the pit plugs of marine and freshwater *Hildenbrandia* populations both have one cap layer, indicating that the two groups most likely share at least common ancestry.

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#### **CODIUM, A GENUS OF CHALLENGE AND FRUSTRATION**

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Although the coenocytic green algal genus *Codium* is represented in almost every local flora, its morphological and taxonomic complexity is not widely appreciated. Ironically, the best known entity is *C. fragile* subsp. *tomentosoides* (van Goor) Silva, a widespread weed that probably represents a single genotype. The diversity of *Codium* continues to be revealed by ongoing studies of new collections. For example, the number of species recognized in the Hawaiian Islands has increased from 6 in 1952 to 15 today. About 50 (nearly half) of all species of *Codium* are clearcut, while the remainder are members of

complexes with a broad range of morphological variability. The unifying ontogenetic event is the production of a palisade of photosynthetic structures (utricles) by enlargement of branches of a sympodial filament. Variations on this theme have arisen phylogenetically by progressive shortening of the filament between utricles to the point that new utricles bud off directly from existing utricles. Several fundamental questions are suggested by the interplay between this filamentous structure and the spectrum of species-specific macroscopic forms. For example, what is the scientific bases that cause the independent primary filaments of a juvenile *Codium* to cooperate and start producing a discrete thallus? What causes a group of apical sympodial filaments to form two groups, one to the left and one to the right, thus effecting dichotomous branching? *Codium* offers challenges for sophisticated research in many fields, including systematics, but the great difficulty in culturing it in the laboratory and in analyzing the taxonomic complexes is frustrating.

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#### **MOLECULAR PHYLOGENETIC POSITION OF CHAETOSPHAERIDIUM GLOBOSUM (CHAROPHYCEAE, CHLOROPHYTA)**

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We sequenced the 18S rDNA of *Chaetosphaeridium globosum* (Nordst.) Klebahn, a microscopic green freshwater epiphyte, in order to assess its phylogenetic affinities in the Chlorophyta. Cultures were harvested after overnight air drying at -18 °C and DNA was extracted and purified using either chloroform and Nucleon Phytopure silica, or the Qiagen gel purification method. Many different PCR primer combinations were tested but only two combinations yielded a product that could be sequenced using dye termination with the ABI 377 sequencer. A phylogenetic analysis of a broad sampling of green algal taxa and *Chaetosphaeridium* (a total of 1681 nucleotide positions were included in our alignment) confirmed that this alga is a member of the Charophyceae (Streptophyta) as earlier microscopical studies had suggested. However, according to our 18S rDNA gene trees and contrary to expectations (based on the ultrastructure of the zoospores and the presence of a unique type of setae) *Chaetosphaeridium* is not closely related to *Coleochaete*. Instead, it represents an early branch in the streptophyte lineage that originates immediately after the emergence of the basal-most charophycean clade, viz. the Charales. *Chaetosphaeridium* is the sister-group of a clade formed

by *Klebsormidium*, *Coleochaete*, *Chlorokybus*, Zygnematales and bryophytes. The node that joins all these taxa has a bootstrap support value ranging between 75 and 100%, depending on the algorithm used and on the taxa included in the analysis. The same tree topology is found irrespective of the tree building algorithm used. No Group I introns are present in the 18S rDNA of *Chaetosphaeridium*.

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**ION FLUXES DURING POTENTIATION AND GAMETE RELEASE IN THE FUCOID ALGA *PELVETIA COMPRESSA* (J. AGARDH) DE TONI.**

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Fucoid algae achieve high fertilization success by synchronous gamete release after potentiation in light and low water motion. In *Pelvetia compressa*, massive release is triggered by darkness. We used X-ray microanalysis of hydrated, rapidly-frozen receptacles to investigate whether osmomechanical forces are involved in gamete release. Receptacles were frozen with LN<sub>2</sub>-cooled copper pliers and held in liquid nitrogen until planed to a smooth face in a cryo-microtome, then examined at -170 °C. When potentiated, all cells except those of the epidermis and mature oogonia had accumulated high K<sup>+</sup> levels (up to 1.2 M in hair cells). Cl<sup>-</sup> was distributed evenly between cells and the extracellular matrix in both medulla and cortex. An efflux of K<sup>+</sup> from hair cell filaments occurred within the first 2 to 5 min of darkness. This appeared to occur as a wave, beginning in distal portions of filaments near the pore and ending in proximal portions at the base of the conceptacle. Simultaneously, K<sup>+</sup> efflux was observed from the cortical and medullary cells into the extracellular matrix. The pattern of Cl<sup>-</sup> flux was similar to that of K<sup>+</sup>. The pre-dark distribution of K<sup>+</sup> and Cl<sup>-</sup> was restored by the beginning of actual gamete release (6-9 min darkness). We propose the following model: 1) Membrane depolarization develops as a downstream effect of the onset of darkness in potentiated receptacles; 2) K<sup>+</sup> efflux into the extracellular matrix causes water uptake and rapid swelling of the gel; 3) gametangia are detached and released by mechanical force during gel transformation.

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**INTRASPECIFIC VARIATION AMONG CULTURES, PREBLOOM, AND BLOOM**

**SAMPLES OF THE BROWN TIDE MICROORGANISM, *AUREOCOCCUS ANOPHAGEFFERENS***

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During the past decade blooms of the brown tide microalga, *Aureococcus anophagefferens*, have occurred sporadically in Peconic and Gardiners Bay in eastern Long Island, NY and in Great South Bay along the south shore of Long Island. Blooms of brown tide vary annually in the timing of their onset, duration and intensity. Additionally, spatial heterogeneity in the onset of blooms is seen among these sites. We hypothesize that temporal and spatial variability in bloom characteristics is due to underlying genetic variation among populations of *A. anophagefferens*. This hypothesis was tested by sequence analysis of the internal transcribed spacer region (ITS) of rDNA. *A. anophagefferens*- specific PCR primers were developed and used to amplify brown tide ITS sequences directly from water samples and laboratory cultures. PCR products from individual water samples were cloned in pCR2.1 and sequenced. Sequence results from up to 20 clones/water sample showed that there are high levels of DNA sequence variation among prebloom and bloom water samples collected in 1995 and 1997. Genetic variation in the ITS region was also observed within single laboratory cultures of *A. anophagefferens*. These results indicate that genetic variation is high both in environmental samples and in individual laboratory cultures and suggest that this variability may be important in controlling the onset and duration of brown tide blooms. Additionally, this genetic variation should be considered when conducting physiological experiments on these laboratory cultures.

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**CONCHOCELIS PHYSIOLOGICAL ECOLOGY OF SIBLING PORPHYRA SPECIES FROM NORTH AMERICA**

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The conchocelis stages of two pairs of putative sibling species of the red seaweed *Porphyra* were compared in laboratory growth experiments. The pairs were

*Porphyra amplissima*-*Porphyra cuneiformis* and *Porphyra linearis*-*Porphyra pseudolinearis*. The first named species of each pair is considered to be North Atlantic species and the latter are North Pacific species. Each pair of sibling species was monitored for growth under varying conditions of light intensity and temperature using a gradient table. Light values tested were 10, 20 and 40  $\mu\text{moles photons}^{-1} \text{m}^{-2} \text{s}^{-1}$ . Four temperatures of 5, 10, 15 and 20 °C were tested. For both pairs the North Pacific species grew much faster initially. The temperature for maximum growth was 10 °C for *P. pseudolinearis* at all light intensities, and 15 °C for *P. linearis*. *Porphyra amplissima* and *P. cuneiformis* both showed optimal growth at 10 °C. There were differences in response to light intensity between the North Atlantic and North Pacific species. Growth of the North Atlantic species appeared to be independent of light intensity. The morphology of *P. pseudolinearis* was dependent on environmental parameters. At 15 °C the habit of the conchocelis of this species became compact and elongate. None of the species became conchosporangial under the test conditions.

**138****CORALLINE ALGAL REEFS: COMMUNITY DYNAMICS STRUCTURED BY SUBSTRATE OR STRUCTURE?**

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This study investigated the influence of coastal coralline populations to the structuring of nearshore subtidal ecosystems through the addition of coralline structure. Unattached species of coralline algae, *Lithophyllum margaritae* and *Neogoniolithon* sp. are widely distributed in the nearshore coastal environments in the Gulf of California, Mexico. Better known as rhodoliths, these algae form extensive beds of a living coralline matrix over soft sandy bottoms that cover up to 100% of the seafloor. These beds are dynamic and can be relocated by currents and storms. Population size increases by 'reproduction' via fragmentation and spore settlement and decreases from mortality primarily due to physical disturbance. Preliminary field growth rates measured from spore settlement experiments result in average growth rates in *Neogoniolithon* sp. of 5.11 mm year<sup>-1</sup>. Development of conceptacles from newly settled spores were observed within a 9 month settlement experiment. These algal beds enhance nearshore ecosystems by adding available structure of a settlement enhancing coralline surface. Species richness is

significantly greater inside these beds in contrast to adjacent sandy flats. Lab and field experiments investigated the mechanism of increased invertebrate species richness by examining larval settlement preferences. Greater settlement of bivalve larvae on living rather than dead coralline surfaces suggest that living coralline substrates may enhance subtidal mollusk populations. Associated organisms may also depend on the presence coralline structure and substrate.

**139****WHY RED ALGAE LACK TRUE TISSUE DEVELOPMENT: A FUNCTIONAL AND EVOLUTIONARY HYPOTHESIS**

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Despite their great antiquity based on fossil and molecular evidence, and their broad diversity in morphological forms and life histories, the red algae never evolved true tissue differentiation. The functional and evolutionary bases for this feature of the Rhodophyta remain largely unexplored. In seeking molecular correlates with developmental complexity, we performed sequence analyses on a gene required for the expression of all nuclear encoded proteins. Here we report comparisons of the 3' distal sequences of the gene encoding the largest subunit of RNA polymerase II (RPB1) from two divergent rhodophytes, *Porphyra yezoensis* Ueda and *Bonnemaisonia hamifera* Heriot. In multicellular green plants and animals this region of the RPB1 gene encodes the C-terminal domain (CTD) that acts as an organizing center for much of the complex control over mRNA transcription that is needed for the differentiation and development of complex tissues. A CTD is not present in either red algal sequence. The phylogenetic and functional implications for this difference in red algal polymerase II will be discussed.

**140****OKADAIC ACID AND ITS ECOLOGICAL ROLE IN THE BENTHIC DINOFLAGELLATE COMMUNITY**

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Serine/threonine protein phosphatases are regulatory enzymes critical to growth and replication in eukaryotes.

Okadaic acid (OA), a potent ser/thr protein phosphatase inhibitor has been well studied in vertebrates. However, little is known about the role it plays in the ecology of its producer, the benthic dinoflagellate *Prorocentrum lima*. The frequency and diversity of toxins produced by benthic dinoflagellates suggests that toxin production may play a role in competition between algal species. To assess the role of OA in growth competition, individual cultures of dinoflagellate species which co-occur with *P. lima* were grown in medium preconditioned by *P. lima*. We found that exudates from *P. lima* inhibited the growth of three of these species. In vitro protein phosphatase assays demonstrated that all four dinoflagellate species possess protein phosphatases which are inhibited by exogenous OA at concentrations similar to mammalian systems (IC<sub>50</sub> ~ 5nM). When treated in culture for 24 hours with either purified OA or medium preconditioned by *P. lima*, dinoflagellate protein phosphatase activity was substantially inhibited. However, *P. lima* preconditioned medium (~ 5nM OA) had greater growth inhibitory action than even 1uM OA. To determine whether its growth inhibitory action was independent of its phosphatase inhibitory activity, the medium was fractionated by HPLC and the fractions were tested for the ability to inhibit growth and protein phosphatase activity. We found that the two actions do not co-elute. These results suggest that although OA has growth inhibitory potential against other microalgae, it does not appear to serve an allelopathic function against co-occurring dinoflagellates.

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#### MOLECULAR PHYLOGENETIC ANALYSIS OF *ULVA* AND *ENTEROMORPHA* (ULVALES, CHLOROPHYTA).

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We inferred phylogeny from comparisons of DNA sequence data among selected members of the Ulvales including *Ulva* and *Enteromorpha*. Both *Ulva* and *Enteromorpha* are important indicators of eutrophication in marine and estuarine ecosystems; further, they are also significant fouling seaweeds. Their inter- and intragenetic classifications are both controversial and questionable, primarily, due to the lack of reliable taxonomic characters. The two objectives of this study were 1) to determine the range of *Enteromorpha* species responsible for causing substantial seasonal algal mats (green tides) in the Ythan estuary, Aberdeenshire, Scotland, and 2) to infer phylogeny among selected

species of *Ulva* and *Enteromorpha*. Sequence analysis of complete ITS 1 & 2 sequence data revealed 5 well supported species complexes in the Ythan estuary, morphologically identified as *E. intestinalis*, *E. compressa*/*E. usneoides*, *E. prolifera*, *E. linza* and *E. pseudolinza*. Furthermore, the inferred phylogenies suggested that neither *Ulva* nor *Enteromorpha* is monophyletic; thus, suggesting that thallus morphology is not a valid character to distinguish the two genera.

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#### *CHATTONELLA SUBSALSA* (RAPHIDOPHYCEAE), A POTENTIALLY HARMFUL ALGA IN THE SALTON SEA, CALIFORNIA

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The Salton Sea, the largest lake in California, with a salinity of approximately 46 g L<sup>-1</sup>, has been subject to large die-offs of both birds and fish. In 1997, the raphidophyte *Chattonella subsalsa* was detected in plankton samples from the Salton Sea and its identity confirmed by transmission electron microscopy. *Chattonella* was present at least from April 15, to November 25, 1997 with maximal densities of 434 cells/ml occurring on June 3. The appearance of *Chattonella* seems to be related to the water temperature reaching approximately 20 °C. Midlake surface temperatures reached a maximum of 34.3 °C on Sept. 6, 1997 and declined to about 20 °C on Nov. 25, 1997, at which time *Chattonella* densities also declined. At the height of the algal population increase in June 1997, *Tilapia* fish gills examined by scanning electron microscopy showed extremely swollen tips of filaments and fused second lamellae. *Tilapia* observed at the Sea during this time period appeared to be "gulping" at the water surface prior to death. Algae in the genus *Chattonella* have caused fish mortality in Japan, Tasmania, Algeria and the Malabar coast of India. In 1997, major fish kills occurred in January-February, May and September at the Salton Sea. *Chattonella* was present at high concentrations for the latter two fish kills and may be implicated as the causative agent.

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#### OPTIMIZATION OF MICROALGAL PRODUCTIVITY OUTDOORS: USE OF AN ON-LINE CHLOROPHYLL FLUORESCENCE TECHNIQUE

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The increasing interest in algal biotechnology for the production of high value products prompts the application of on-line measurements to monitor and control the growth parameters and to get rapid evidence of unfavorable conditions affecting the performance of the culture. Since environmental stress affects the function of photosystem II (PSII), directly or indirectly, the use of chlorophyll fluorescence can represent a useful tool to investigate the stress response mechanism and to quantify the effect of stress on the productivity of the organism growing under rapidly changing environmental conditions such as those usually found outdoors. An on-line chlorophyll fluorescence technique, based on the saturating pulse method to assess the performance of the outdoor culture of the cyanobacterium *Spirulina* grown in closed tubular photobioreactors, has been developed and tested. Diurnal changes in maximum photochemical yield (Fv/Fm) and effective quantum yield of PSII were measured in outdoor cultures grown under different stress conditions (e.g. low temperature, high oxygen concentration, high oxygen concentration plus low temperature), using a portable pulse-amplitude-fluorometer. The results indicate that changes in the biomass yield of the cultures correlate well with changes in the daily integrated value of the estimated electron transport rate (ETR) through the PSII ( $dF/F'm \times \text{Photon Flux Density}$ ). ETR has proved to be a simple and reliable parameter to be used in estimating the photosynthetic performance of outdoor cultures of *Spirulina* and to reach rapid decisions concerning their management.

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### **CELL CYCLE REGULATION IN THE FLORIDA RED TIDE DINOFLAGELLATE, *GYMNO-DINIUM BREVE***

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Many dinoflagellates display phased cell division, in which mitosis is restricted to a narrow window of time during either the light or dark phase of the diel cycle. This circadian regulation may impose a maximum division rate of 1 division day<sup>-1</sup>. Thus, an unresolved question is whether dinoflagellate blooms result from events which trigger explosive growth rates, or if cells replicate at a relatively constant rate, with blooms resulting from advection and subsequent maintenance of dense cell populations by physical or biological

processes. The cellular mechanisms which mediate phased cell division are not understood, nor are mechanisms known which might release cells from this circadian control to result in 'explosive' rates of growth. In this study we sought to identify mechanisms regulating the cell cycle in the Florida red tide dinoflagellate, *Gymnodinium breve*. Growth rates of 0.2 to 0.5 division day<sup>-1</sup> observed in both laboratory and field populations of *G. breve* are not sufficient to account for its dominance in the water column. In both laboratory and field populations, we found that the cell cycle of *G. breve* is phased to the diel cycle, with S-phase beginning during the light phase and mitosis occurring during the dark. The dark/light transition appears to serve as the entraining signal. *G. breve* possesses the eukaryotic cell cycle regulator, cyclin dependent kinase (CDK), as identified by immunoblotting and reversible inhibition of cell cycle progression by olomoucine, a specific inhibitor of CDKs. Current work on signaling events which regulate cell cycle progression will be discussed.

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### **PLASMA MEMBRANE FLUIDITY ANALYZED IN CELLS EXPOSED TO VOLATILE AROMATIC HYDROCARBONS USING THE FLUOROPROBE DIPHENYLHEXATRIENE**

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Crude oil is toxic to phytoplankton. The most toxic components are volatile aromatic hydrocarbons (VAH; benzene, toluene, xylene, etc.). However, mechanisms of toxicity at the molecular level are not clear. We have previously reported data supporting our hypothesis that toxicity of VAH is due to disruption of optimal physical state of cytoplasmic membranes. These data suggest that VAH act as lipid solvents, altering fluidity of cytoplasmic membranes. We now present more direct evidence that changes in membrane fluidity occur during VAH exposure. The fluorescent probe diphenylhexatriene (DPH) was utilized. Optimal conditions were determined for entry of DPH into plasma membranes of intact microalgal cells. Subsequently, cells pre-incubated with DPH were exposed to increasing concentrations of various VAH. Our data indicate that with increasing concentrations of VAH, membrane fluidity increases in a dose-dependent manner. However, VAH concentrations required to elicit changes in membrane fluidity as detected by DPH

fluorescence were greater than those VAH concentrations previously shown to produce changes in membrane lipid (fatty acid) composition. These experiments provide clear evidence showing one mechanism of VAH toxicity is disruption of the structural integrity of plasma membranes. A model will be introduced incorporating data from all these experiments serving to illustrate the toxic mechanism and the means by which algal cells adapt to sub-lethal VAH exposure.

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**A CRITICAL APPRAISAL ON SOME TRADITIONAL CRITERIA AND CURRENT PROCEDURES IN THE TAXONOMIC TREATMENT OF OEDOGONIALES (CHLOROPHYCEAE)**

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Identification of taxa within the Oedogoniales is largely based on morphology of sexually-mature reproductive structures. Taxonomic criteria were established during the nineteenth century, and generalized in their use after Hirn's (1900) monograph, the nomenclatural point of departure of the order. However, the validity of these characters currently considered of taxonomic value has not been a subject of reviewing up to date. In this work a critical appraisal based on culture and ultrastructure evidence derived from the study of twenty-four taxa is presented. The following characters are considered: 1) number of oogonia in series, 2) type and position of oogonia opening, 3) apical cell morphology, 4) types of dwarf males, and 5) vegetative cell morphology. Additionally, the fact that many uncomplete descriptions of taxa could be the result of occurrence in nature of female apomictic populations (species?) is vindicated. Future and perspectives of systematics study of the Oedogoniales is discussed.

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**A COMPARISON OF ALGAL DIVERSITY AMONG POLLUTED, RESTORED AND NON-POLLUTED STREAMS IN SOUTHEASTERN OHIO**

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Ten streams along a gradient from acid mine drainage (AMD) (3 to 3.1 pH) to pristine (6.7 to 7.1 pH) were

examined on a monthly basis for one year. Physical and chemical properties of the streams were measured including pH, conductance, metal concentration, average current velocity, width and depth. Macroalgal taxa were collected and percent cover estimated. Diatoms were sampled from the riffle area and subsequently identified and enumerated. A dendrogram of the physical and chemical properties showed the three AMD impacted streams grouped together and the five slightly impacted streams grouped with the two pristine streams sampled. Principal components analysis (PCA) revealed pH, conductance, aluminum and silica to be the most influential parameters separating the severely impacted AMD streams from the other sites. Among the low impact and pristine sites, phosphorus and maximum stream width played the greatest role in grouping of streams. AMD streams had either no macroalgae or were dominated by *Klebsormidium* sp., which appears to be an indicator of poor water quality. The other streams varied in macroalgal species richness with season and water quality. Diatom taxa were fewest in AMD streams. Species richness was highest in streams draining reclaimed coal mines. The pristine streams were intermediate. It appears that the diatom species richness in streams along this gradient, AMD - reclaimed - pristine, support the intermediate disturbance hypothesis.

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**A MOLECULAR INVESTIGATION OF THE SYSTEMATIC RELATIONSHIP AMONG SIRODOTIA SPECIES (BATRACHOSPERMALES, RHODOPHYTA) IN NORTH AMERICA.**

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Three morphologically delineated *Sirodotia* species, *S. suecica*, *S. tenuissima* and *S. huillensis*, are recognized in North America. The rDNA internal transcribed spacer (ITS), RUBISCO large subunit (rbcL) and rbcL-S spacer DNA sequence were employed to investigate the systematic relationships among these species. A total of six populations were sampled comprising the following: *S. suecica* from Rhode Island and Labrador, *S. tenuissima* from Rhode Island and *S. huillensis* from Texas, Mexico and Costa Rica. The sequence data for the nuclear and plastid DNA revealed little variation (< 1% sequences divergence) among the *S. suecica* and *S. tenuissima* populations. This result suggests that *S.*

*suecica* and *S. tenuissima* are conspecific and morphological characters used to distinguish these species are not phylogenetically informative. Sequence variation among *S. huillensis* populations was < 1% in the plastid DNA sequences. Between *S. suecica* and *S. huillensis* there was 5% divergence in the combined *rbcl* and spacer region. ITS sequence data for populations within *S. huillensis* was variable. In addition, the ITS sequences between *S. huillensis* and *S. suecica* diverged significantly. From this research we conclude that *S. huillensis* and *S. suecica* are valid species.

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#### THE PH TOLERANCE OF *CHLAMYDOMONAS APPLANATA* AND *CHLAMYDOMONAS ACIDOPHILA* (VOLVOCALES, CHLOROPHYTA)

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The effects of H<sup>+</sup> ions on the growth and ultrastructure of two chlamydomonads were examined. Growth was determined by measurements of the optical density, while cytological changes were quantified via morphometric analysis of electron micrographs. Both species exhibited wide tolerance growing at pH values ranging from 8.4 to 3.4. Optimum growth was observed at 8.4 to 6.4. At pH 4.4, *Chlamydomonas applanata* cell size decreased. At pH 3.4 the effects were quite dramatic and included cell death, formation of large palmelloid colonies, loss of motility, abnormal cell division, increase in the volume of the vacuome and appearance of akinetes. *C. acidophila* cells increased in volume at both pH 4.4 and 3.4. Exposure to the latter H<sup>+</sup> ion concentration resulted in inhibition of autospore release and a significant decrease in chloroplast volume accompanied by an increase in vacuolar volume. At this pH value cellular contents appear darker and increased autolysis is apparent. The implications of these results are discussed. Furthermore, possible mechanisms of tolerance are investigated.

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#### MASS CULTURING OF MICROALGAE; FROM OPEN PONDS TO CLOSED SYSTEMS

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In the attempt to design a cost effective culturing system suitable for outdoor mass culturing of microalgal most of the producers use shallow ponds intensively mixed

by paddle wheels and lined by some kind of a plastic liner. In the case of *Spirulina* the ponds are raceway shaped and vary in size from 0.1 to 0.5 Ha. The *Chlorella* ponds are round with distinct mixing devices and deeper than the *Spirulina* ponds. The intensive production of *Dunaliella* is carried out using similar ponds to the one developed for the *Spirulina* production, while most of the *Dunaliella* production is carried out in large deep lagoons in an extensive mode of production. It is most likely that each solution represents a trade-off between the special requirements of the production system, the know-how available to the producers at the time of construction and the local costs of the different components. Yet it is clear that the open pond represent a very limited solution for the need of this developing biotechnology so as to enable growing more species of algae at a more diverse environment and at a better controlled system. The future of algal biotechnology rests, to a large extent, on two factors: a) The ability to reduce costs of production. b) The development of suitable closed photobioreactors.

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#### GREEN TIDE ALGAE OF THE PADILLA BAY ESTUARY, WASHINGTON

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Chlorophytic macroalgae were collected in August and September 1996 and April through August 1997 at six sites in the Padilla Bay Estuary of Washington State and identified to species using morphological and molecular characters. Based on morphological identification, fourteen chlorophytic macroalgal species were found, including representatives of *Acrosiphonia*, *Blidingia*, *Enteromorpha*, *Rhizoclonium*, *Ulva*, *Ulvaria* and *Urospora*. A different assemblage of species was found among and within sites during each collection while the number of species at all sites peaked in May and June 1997. Preliminary molecular analysis using restriction digests of the 5.8s coding region and flanking internal transcribed spacers of nuclear rDNA was carried out on individuals of *Enteromorpha prolifera*, *Enteromorpha linza* and *Ulva fenestrata*. Morphological and molecular data are consistent for *E. prolifera* individuals, but are inconsistent for *E. linza* and *U. fenestrata* individuals. Digestion patterns suggest that gene flow may be occurring among these species and raises questions about the current taxonomy and systematics of Ulvaceae. These questions will be explored in a cladistic analysis using DNA sequence data from introns in chloroplast *trnL* and nuclear RNA Polymerase II genes.

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**THE *EUGLENA* PHOTORECEPTOR: A 3D CRYSTAL OF AN OPTICALLY BISTABLE INTEGRAL MEMBRANE PROTEIN**

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The naturally occurring crystalline photoreceptor of *Euglena* represents perhaps one of the most intriguing structures in biology. This ancient eukaryote uses a simple, yet sophisticated system as a light detector—a single locomotory flagellum consisting of an axoneme, a paraxial rod and a basal paraflagellar swelling (the photoreceptor) which lies in a reservoir adjacent to an orange-red cytoplasmic stigma. Earlier, Gualtieri suggested that the *Euglena* photoreceptor is a crystal of single membrane proteins and interpreted the structure as a 3D crystal, which we have since shown to have dimensions of 1.0 x 1.5 x 0.7  $\mu\text{m}$  and have confirmed its structure by freeze-fracture electron microscopy. The dimensions of the monoclinic unit cell of a single crystalline sheet are ca. 50  $\text{\AA}$  x 40  $\text{\AA}$ , and the height of the integral membrane protein is ca. 70  $\text{\AA}$ . Since no symmetry but the inversion is observed in the Fourier transform, the plane group of the crystal sheet should be P1<sub>1</sub>, and the 3D cell appears to be triclinic. The transmembrane protein is suggested to be rhodopsin-like, and recent data show that an antibody against recombinant opsin recognizes a 30 kd protein in the *Euglena* photoreceptor. Our data further show that this rhodopsin undergoes light conformational changes, i.e., repeated and reversible fluorescence changes with a determinate kinetics, results suggesting that the protein possesses optical bistability. The very high quantum yields of the forward and reverse reactions are nearly identical and close to unity.

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**PHRAGMOPLAST-MEDIATED CYTOKINESIS IN *TRENTEPOHLIA ODORATA* (CHLORO-PHYTA): AN IMMUNOFLUORESCENT CYTO-LOGICAL STUDY**

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Molecular studies have shown that green algae and land plants share a common ancestor, and charophycean green algae are the closest relatives of land plants. Some charophycean algae have a phragmoplast-mediated cytokinesis, the type basic to all land plants but not present in most other green algae. Inexplicably, the phragmoplast-type-cell division has been documented, via transmission electron microscopy in *Cephaleuros parasiticus* Karsten, a trentepohlean alga. Molecular data indicate that the Trentepohliales (subaerial green algae) are not in the charophycean lineage, but rather are in or are very closely related to the Ulvophyceae (Chlorophyta). The present study was undertaken to determine if the appearance of a phragmoplast in *Cephaleuros* represents an anomaly in the trentepohlean group. Immunofluorescent cytological techniques were used to document cell division in *Trentepohlia odorata* (Wiggers) Wittrock. This study clearly revealed a phragmoplast-type cell division in *T. odorata*, thus offering further proof that this type of division is characteristic of the Trentepohliales. The evolutionary significance of phragmoplast-mediated cell division in higher plants has been appreciated for many decades. Further investigation of the little-studied Trentepohliales, which represent the conundrum of "chlorophyte" algae having "charophyte" cell division, might provide a key to unraveling the evolutionary history and development of the diverse microtubular systems in the fundamental process of cytokinesis, information which is crucial to a full understanding of green plant evolution.

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**CAN THE AQUACULTURED, NON-INDIGENOUS RED ALGA, *PORPHYRA YEZOENSIS*, RECRUIT IN EASTPORT, MAINE?**

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Coastal Plantations International is in its 8th year of commercially farming *Porphyra yezoensis*, an introduced species of nori, in Cobscook Bay, Maine. A preliminary survey found some limited evidence of *P. yezoensis* recruitment at a farm site during the growing season, but no evidence of over-wintering. The current study examines the potential ecological impact of farming *P. yezoensis* at a second site. Surveys of the intertidal area surrounding this site for *P. yezoensis* recruitment and survival are being conducted using artificial (synthetic netting used in the commercial growing of nori) and natural substrates (tagged macroalgae for epiphytic settlement and 10 meter

transects of the substrata). *Porphyra* species are being identified by microscopic examination and isoenzyme electrophoresis. *P. yezoensis* has not been positively identified in any transect samples to date. Five putative *P. yezoensis* were identified on the artificial substrates collected in November. Four of these potential exotics were recruited at a single location, and this site effect is being further investigated by tracking water flow patterns. In November, strands of netting seeded with *P. yezoensis* were attached to artificial substrates and allowed to overwinter. In March, *P. yezoensis* was not found on the artificial substrates or the seeded strands. The inability of *P. yezoensis* to overwinter is being investigated in laboratory freezing tolerance experiments. The potential for competition between *P. yezoensis* and local *Porphyra* species is being evaluated through measurements of photosynthetic capacity and comparative growth studies.

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**PHENOLOGICAL AND LIFE HISTORY STUDIES OF *ANTITHAMNIONELLA FLOCCOSA* (CERAMIACEAE, RHODOPHYTA) FROM NEWFOUNDLAND**

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*Antithamnionella floccosa* is widely distributed in both the eastern and western North Atlantic. In Newfoundland, it is commonly attached to stable rocky substrates in the immediate subtidal. Detailed phenological studies show its presence throughout the year, but with considerable die back in August and September. In some localities, and some years, only sterile and tetrasporophyte plants are found. Other populations show that fertile male gametophytes and female gametophytes bearing carposporophytes occur predominantly between November and February. Fertile tetrasporophytes show a seasonally bimodal distribution, being fertile predominantly between October and March, and in June and August. At other times, sterile plants predominate. Cytological and culture studies suggest that although the species has a basic *Polysiphonia*-type life cycle, vegetative reproduction and perennation are common. It also seems that meiosis can frequently fail and tetrasporophytes may regenerate tetrasporophytes. Summer temperatures appear restrictive with little or no growth occurring appearing above 15 °C.

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**MARINE SEDIMENT STABILIZATION BY MICROORGANISMS : THERE'S MORE TO IT THAN MEETS THE EYE!**

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The role of microorganisms in the mechanical stabilization of marine littoral sediments is not clear. Previous investigations have highlighted either the role of bacteria to the exclusion of the algal contribution, or vice versa. Since both bacteria and algae [notably diatoms] are abundant in these sediments and both are known to produce potentially sediment particle - binding extracellular macromolecules, it is not likely that the contribution of one or the other can be discounted. However, what is more interesting to the phycologist is that the role of diatoms in the stabilization process also is not clearly understood. Most studies have involved a group of undefined organisms in their natural setting or have utilized a single organism in a laboratory simulation. Our work, using several axenic cultures isolated recently from False Bay, San Juan Is., WA, shows that the concept that all diatoms from the same environment make similar contributions to the stabilization process is false. Motile species of diatoms [epipelagic] are assumed to contribute to sediment stabilization as a result of their secretion of a motility polymer which binds the particles together to form aggregates and the non- motile species [epibenthic] by making bridging polymers. We find that no such distinction exists. While motility is fundamentally a stochastic process, we have shown that this phenomenon can be a directed response [e.g. cellular dispersal] that is probably the result of cell-cell signaling and is dependent on the growth history of the cells [temperature, light level].

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**THE ROLE OF SUSPENDED SEDIMENT ON CELL MORPHOMETRY IN *CLADOPHORA GLOMERATA* IN THE COLORADO RIVER, USA**

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We examined the role of suspended sediments on the cell morphology of *Cladophora glomerata* in the Colorado River through Grand Canyon National Park. *Cladophora* and its highly branched filaments provide large surface areas for the colonization of epiphytes and a refugium for invertebrates. Suspended sediment loads

delivered into the Colorado River via tributaries produce variable turbidities along the Colorado River. Turbidity changed from 0.5 NTU at Lees Ferry (Rkm 0.0) to 7.9 NTU at Gorilla Island (Rkm 352). We found that elevated sediment loads influenced cell morphology of *C. glomerata* along turbidity gradients. Primary branches showed a significant decrease in cell lengths from 993  $\mu\text{m}$  (SE# 21) to 814  $\mu\text{m}$  (# 23) between the clear water habitat at Lees Ferry and the downstream turbid water habitat of Gorilla Island, while cell widths increased from 88  $\mu\text{m}$  (# 1.5) to 95  $\mu\text{m}$  (# 2). We tested the effects of suspended sediments and light on the cell morphology of *C. glomerata* in the field. Cobbles colonized with *C. glomerata* were translocated from Lees Ferry to a turbid water zone in the Paria River. Cells showed a significant decrease (30%) in length after one month. In contrast, *in situ* experiments in reduced light at Lees Ferry without suspended sediments showed no significant changes in *C. glomerata* cell morphology. We propose that modifications in cell dimensions strengthen filament structure and reduce shear stress in high-suspended sediment environments, reducing surface area for epiphytic diatoms. The loss of surface area for epiphytic growth may have important trophic implications in the Colorado River foodweb.

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#### **RHODOLITH GROWTH FORMS AND SPECIES DISTRIBUTION IN THE GULF OF CALIFORNIA, MEXICO**

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Rhodolith beds in the Gulf of California are composed of three species and several growth-forms. We evaluated the species and growth-form composition of rhodoliths in the two most common environments where beds occur, shallow water areas influenced by primarily by waves and deeper areas influenced by bioturbation and currents. *Neogoniolithon trichotomum* was restricted to shallow beds in the central Gulf, while the other two species were widely distributed with *Lithophyllum margaritae* dominant in deeper, sandy areas and *Lithothamnion crassiusculum* dominant in shallow, rocky areas. Fruticose growth forms were common throughout the Gulf, but the presence and relative abundance of other forms varied among sites. These results suggest that environmental factors such as depth and substrate greatly influence the structure and distribution of rhodoliths in beds in this geographic region.

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#### **EXPERIMENTAL COMMERCIAL CULTIVATION OF INDIGENOUS PORPHYRA SPECIES IN NORTHEAST AMERICA**

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We have embarked upon a study of domesticating indigenous species of *Porphyra* for commercial cultivation with the assistance of the New England and National Sea Grant College programs. Detailed seasonal and spatial collections, from diverse coastal and estuarine habitats, have been made to delineate the seasonality and habitat preferences of six species of *Porphyra* in coastal New England and the Canadian Maritimes (*Porphyra amplissima* (Kjellman) Setchell & Hus in Hus, *P. miniata* (C. Agardh) C. Agardh, *P. umbilicalis* (Linnaeus) J. Agardh, *P. linearis* Greville, *P. purpurea* (Roth) C. Agardh, and *P. leucosticta* Thuret in Le Jolis). At least four different species, *P. leucosticta*, *P. purpurea*, *P. amplissima* and *P. linearis*, are being examined for experimental commercial production. Several strains of each of the species have successfully completed their life cycles in culture and F<sub>2</sub> individuals have been obtained for *P. amplissima*, *P. leucosticta* and *P. purpurea*. Whether or not nori aquaculture will ultimately succeed in New England and the Canadian Maritimes will depend in large part upon several key factors, including: (1) successful transfer and modification of Asian cultivation technologies to local coastal environments; (2) development of genetically improved indigenous American cultivars of marketable nori that will extend the growing and harvest seasons; (3) establishing a constant supply of a "seedstock" of juvenile organisms that will be readily available; (4) the expansion of the area presently used for cultivation (i.e. beyond northern Maine) and (5) integrating nori mariculture with commercially important finfish aquaculture.

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#### **'TASTE/ODOR METYABOLITE'-PRODUCING CYANOBACTERIA WITHIN CATFISH AQUACULTURE SYSTEMS: ABUNDANCE**

**PATTERNS DURING THE SUMMER-FALL TRANSITION**

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The presence of cyanobacteria in pond aquaculture systems can result in economic losses as a result of secondary metabolite formation. These compounds may include toxins as well as compounds that impart a noxious taste to fin and shellfish growing within these ponds. Fifteen channel catfish ponds were monitored weekly to assess algal composition, photo-pigment content (HPLC), and presence of off-flavor compounds (2-methylisoborneol-MIB and geosmin) during October-November 1997. Four of these ponds were also monitored for concentration of off-flavor in the fish population and on one occasion for intra-pond fish concentrations of MIB and microcystin. All ponds had filamentous blue-green taxa as dominants at the onset of sampling; coccoid cyanobacteria and diatoms replaced these as cooler weather approached. Pigment data also indicated a replacement of zeaxanthin-containing cyanobacteria with myxoxanthophyll-containing forms, as well as increased fucoxanthin (a diagnostic pigment for diatoms). Off-flavor concentrations in fish better coincided better with water concentrations of geosmin ( $r = 0.87$ ) than MIB ( $r = 0.60$ ) during this period. MIB in fish from one pond was highly variable, with coefficient of variation exceeding 30% of mean ( $n = 21$ ). No evidence of microcystin damage was evident from liver analyses of these fish.