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## FATTY ACIDS IN PHOTOTROPHIC AND MIXOTROPHIC *GYRODINIUM GALATHEANUM* (DINOPHYCEAE)

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Fatty acids were measured in *G. galatheanum* grown either phototrophically, or mixotrophically with *Stoeratala major* (Cryptophyceae) as prey. *G. galatheanum*, like many photosynthetic dinoflagellates, contains high amounts of n-3 long-chain-polyunsaturated fatty acids (LC-PUFA) such as docosahexaenoic acid (DHA, 22:6n-3) and the hemolytic toxic fatty acid 18:5n-3. We hypothesize that a benefit of phagotrophy in *G. galatheanum* is the acquisition of precursor linolenic acid (18:3n-3) that fuels LC-PUFA synthesis. Phototrophs grew at 0.37 d<sup>-1</sup>, while mixotrophs grew at 0.40 d<sup>-1</sup> with a feeding rate of 0.62 d<sup>-1</sup>. Photosynthesis was lower in mixotrophs (3.7 pg C cell<sup>-1</sup> h<sup>-1</sup>) than phototrophs (4.9 pg C cell<sup>-1</sup> h<sup>-1</sup>). DHA levels were higher in mixotrophs [3.7 (+/- 0.11) pg cell<sup>-1</sup>] than phototrophs [3.0 (+/- 0.16) pg cell<sup>-1</sup>] and prey [0.4 (+/- 0.01) pg cell<sup>-1</sup>]. 18:5n-3 levels [1.7 (+/- 0.03) pg cell<sup>-1</sup>] were similar in phototrophs and mixotrophs. An intermediate in n-3 LC-PUFA synthesis, 20:4n-3, accumulated in mixotrophs [0.6 (+/- 0.27) pg cell<sup>-1</sup>] relative to phototrophs (not detected) and prey [0.03 (+/- 0.002) pg cell<sup>-1</sup>]. Low ratios of linolenic acid to DHA in phototrophic *G. galatheanum* (0.14) relative to mixotrophic *G. galatheanum* (0.29) and prey (2.14) are consistent with substrate limitation of LC-PUFA synthesis in phototrophs. Accumulation of 20:4n-3 suggests incomplete conversion of linolenic acid to DHA, possibly due to conditions in batch culture. We conclude that precursors for n-3 LC-PUFA biosynthesis in *G. galatheanum* may be acquired through ingestion of *S. major*, and may partially control feeding/photosynthesis in mixotrophic populations.

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## SPECIES COMPOSITION AND SEASONAL PERIODICITY OF MACROALGAL SPECIES IN CORPUS CHRISTI BAY, TEXAS

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Benthic algal sampling from selected sites along Corpus Christi Bay and from one site at the Port Aransas,

Texas south jetty was completed between April 1999 and February, 2000. Species composition, seasonal periodicity, and fluctuations in temperature and salinity were determined. This is the first comprehensive study of benthic macroalgae conducted in Corpus Christi Bay, which is shallow, turbid, and lacks natural hard substrate. Man-made jetties are necessary for suitable floral attachment. Macroalgae are affected by changes in salinity as freshwater inflows are followed by periods of drought, which increase salinity. These effects are most notable where freshwater enters at the south end near Oso Bay and at the north end at Nueces Bay. Previous Texas algal collections described species of *Enteromorpha*, *Ulva*, *Gelidium*, and *Gracilaria* as the most dominant plants of the area. This supports the current study with the additions of *Hypnea musciformis* and *Centroceras clavulatum*. Dominant plants at the Port Aransas jetty include *Ulva fasciata*, *Padina gymnospora*, and *Hypnea musciformis*. The Rhodophyta including *Gracilaria*, *Gelidium*, and *Centroceras clavulatum* dominate the bay and do so throughout the year. Chlorophytes, although few in species richness, are important to benthic coverage and biomass. Phaeophyta are found predominantly at the Port Aransas jetty with *Sargassum*, *Dictyota dichotoma*, and *Petalonia fascia* being most abundant. A transition occurs in species composition as the water temperature changes seasonally. *Hinckesia*, *Ectocarpus*, and *Petalonia fascia* are found only during the cooler months.

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## NEW AND RARE CHRYSOPHYTES FROM WYOMING AND COLORADO LAKES

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Colorado and Wyoming lakes with high pH values generally ranging from 8 to 10 are dominated by phytoplankton belonging to the chrysophytes, diatoms and cryptomonads. Our studies on phytoplankton diversity in Dowdy, South Delaney Buttes and Cowdrey Lakes in Colorado and Diamond and Twin Buttes Lakes in Wyoming have yielded several isolates of rare or new chrysophytes. These isolates were examined with light microscopy, SEM, TEM, and novel information regarding their structure is presented. Proposed new species belong to the genera *Saccochrysis*, *Prymnesium* and *Chrysochromulina*. Chrysophytes that were examined for the first time include *Monochrysis aphanaster*, *Uroglenopsis*, and a mixotrophic species of *Ochromonas*, which ingests small diatoms. Probable new species are the following: *Saccochrysis* sp. nov. differs in chloroplast structure, cell shape and cell number/colony, and absence of a posterior vacuole; *Prymnesium* sp. nov. is the first freshwater form examined with EM and differs in scale morphology; and *Chrysochromulina* sp. nov. is similar to *Chrysochromulina parva* but lacks scales. The ultrastructure of *M. aphanaster* is

unique and appears to be a member of the Chrysophyceae. It has two flagella, one of which is emergent, while the other is hidden within an invagination of the cytoplasm. *Diacronema* and *Sarcinochrysis* spp. from these lakes also were isolated and reinvestigated, and some unique cellular features will be discussed.

## 4

#### ALGAL COMMUNITY STRUCTURE OF THE EAST AND WEST FLOWER GARDEN BANKS, NORTHWESTERN GULF OF MEXICO

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The algal communities of the Flower Garden Banks National Marine Sanctuary have not been comprehensively evaluated and only a few dominant macroalgal species have been reported. This study utilizes both destructive and non-destructive sampling techniques to characterize and taxonomically identify the 'algal mat' community structure. The East and West Flower Garden Banks are located on the outer continental shelf approximately 200 km off the Texas-Louisiana coastline. The average depth of both banks is 100 m with the crest approximately 20 m from the surface. Harvest and photogrammetric samples were collected during two extended cruises to the Flower Garden Banks in October 1998 and March 1999. Forty, 0.25-m<sup>2</sup> quadrats of standing stock material were randomly collected along with one hundred sixty-one 0.25 m<sup>2</sup> photo-quadrats from an average depth of 27 m. Photo-transparencies were projected to an actual size grid with 25 random points. Four thousand twenty-five transparency points were evaluated and used to calculate percent composition of algal cover. Harvest samples were used to characterize the "algal mat" composition, species richness, abundance, and biomass. Forty-two species were identified from the samples representing 14 Orders. The "red algal mat" was the dominant algal coverage comprising 38.4% of all photogrammetric samples. This mat was primarily composed of members from the Order Ceramiales. *Centroceras*, *Ceramium*, and *Polysiphonia* comprised 33.4% of the mat, *Anotrichium* and *Hypoglossum*, 22.4%.

## 5

#### THE PHYLOGENETIC RELATIONSHIPS OF THE PHAEOTHAMNIOPHYCEAE, WITH COMMENTS ON CERTAIN CAPSOID MEMBERS OF THE CHRYSOPHYCEAE

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The Phaeothamniophyceae was based upon ultra-structure, pigment analysis and *rbcL* gene sequences. Recently we have successfully obtained SSU rDNA sequences from members of this relatively new class. These sequences corroborate the phylogenetic relationship of the Phaeothamniophyceae as a close relative of the Phaeophyceae and Xanthophyceae. Additionally we show that the "walled" *Chrysocapsa epiphytica* is a synonym of *Tetrasporopsis fuscescens* and belongs in the Phaeothamniophyceae. Conversely, "wall-less" taxa such as *Chrysoapsa vernalis*, *Dermatochrysis*, and *Chrysonobula* belong within the Chrysophyceae. Furthermore, we suggest that the capsoid *Tetrachrysis dendroides* is a synonym of *Stichogloea globosa* and belongs within the Phaeothamniophyceae. We will also report new findings for other genera (e.g. *Phaeobotrys*, *Phaeothamnion*, *Phaeoschizochlamys*, *Stichogloea*). It appears that the capsoid habit evolved independently in the Phaeothamniophyceae and the Chrysophyceae, thus providing a new example of convergent evolution for thallus organization.

## 6

#### RELATIVE IMPORTANCE OF RECRUITMENT VERSUS REGENERATION IN MAINTAINING THE POPULATIONS OF *SARGASSUM HENSLOWIANUM* IN PING CHAU, HONG KONG

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Populations *S. henslowianum* dominate the shallow subtidal waters of Ping Chau Island, Hong Kong. A study was carried out to evaluate the relative importance of recruitment vs regeneration from the holdfast in the maintenance of these populations. The number of recruits on clearings, artificial and natural substrata as well as the density of the natural populations were evaluated on a periodic basis from March 1998 to March 2000. The results indicated that although up to 85 to 100% of the individuals of this species may be reproductive during the winter reproductive season (Nov to Feb), only up to a maximum mean density of 17 recruits 0.25 m<sup>-2</sup> were found in the cleared areas or artificial substrata provided. Less than 10% of these eventually survived the following growth season. In the natural stands, new individuals (recruits) constituted only less than 15% of the mean density. The number of new recruits was particularly low around older individuals. Thus, these populations were mainly made up of older individuals regenerating from their perennating holdfasts. It appears that regeneration from the holdfast is a far more impor-

tant strategy than recruitment in the maintenance of these populations.

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### **IN VIVO CHARACTERIZATION OF THE DIATOM MULTIPARTATE PLASTID TARGETING SIGNAL**

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Diatoms and related algae have plastids that are surrounded by four membranes. The outer two membranes are continuous with the endoplasmic reticulum and the inner two membranes are analogous to the plastid envelope membranes of higher plants and green algae. Thus the plastids are completely compartmentalized within the ER membranes. The targeting presequences for nuclear-encoded plastid proteins have two recognizable domains. The first domain is a classic signal sequence, which presumably targets the proteins to the endoplasmic reticulum. The second domain has characteristics of a transit peptide, which targets proteins to the plastids of higher plants. To characterize these targeting domains, the presequence from the nuclear-encoded plastid protein AtpC was utilized. A series of deletions of this presequence were fused to Green Fluorescent Protein (GFP) and transformed into cells of the diatom, *Phaeodactylum tricorutum*. The intracellular localization of GFP was visualized by fluorescence microscopy. This work demonstrates that the first domain of the presequence is responsible for targeting proteins to the ER lumen and is the essential first step in the plastid protein import process. The second domain is responsible to directing proteins from the ER and through the plastid envelope and only a short portion of the transit peptide-like domain is necessary to complete this second processing step. *In vivo* data generated from this study in a fully homologous transformation system has confirmed Gibbs' hypothesis regarding a multistep import process for plastid proteins in chromophytic algae.

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### **THE ROLE OF CARBONIC ANHYDRASE IN THE FORMATION OF MOUGEOTIA (CHAROPHYCEAE) BLOOMS IN ACIDIC ENVIRONMENTS**

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*Mougeotia* sp, a dominant component of a metaphytic algal bloom-forming alga in an experimentally acidified lake (Little Rock Lake, Vilas Co. WI) was isolated to analyze pH effects on carbonic anhydrase (CA) activity, localization, and algal morphology. A potentiometric method was used to assess internal and external CA activities using two CA inhibitors, ethoxzolamide (EZA) and acetazolamide (AZA) in cultures grown at pH 5 and pH 8. External and total CA activities at pH 8 were significantly greater than those at pH 5, but internal CA activities were not significantly different at both pH levels. The non-penetrating inhibitor AZA inhibited external CA activity at pH 8, whereas EZA, which inhibits internal CA activity in some green algae, did not inhibit *Mougeotia* CA activity, possibly because of permeability problems. Ultrastructural immunogold labeling with a polyclonal antibody for *Chlamydomonas* external CA suggested that an antigenically-similar enzyme was located in the periplasmic space and inflated end walls of *Mougeotia* grown at both pHs. This antibody, labeled the *Mougeotia* chloroplast, but not the pyrenoid and cytoplasmic peripheral regions. Activity measurements, inhibitor results, and localization data were consistent with the operation of a carbon concentrating mechanism (CCM) at both pH levels. Growth form, cell dimensions, chloroplast morphology, and cell wall ultrastructure were significantly different in cultures grown at both pH levels. These features probably contributed to the ability of *Mougeotia* to form blooms in acidified waters. A polyclonal antibody to pea chloroplast CA did not label *Mougeotia*, suggesting evolutionarily significant differences in the plastid CA systems of this alga and higher land plants.

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### **THE PRESENCE OF CALLOSE IN THE PRIMARY ZYGOTE WALL OF CHLAMYDOMONAS MONOICA AND THE EFFECTS OF ITS DEGRADATION ON ZYGOTE DEVELOPMENT**

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*Chlamydomonas monoica* constructs a temporary primary wall around its developing zygotes. This study aimed to confirm callose as a component of the primary wall, as well as to note the effects of primary wall degradation on zygote development. Glucanase, specific for the  $\beta$ -1,3 glycosidic bonds comprising callose, was added to mating media at concentrations ranging

from 5 to 1 mg ml<sup>-1</sup> and light microscope observations were made as the zygotes developed. The overall health of the zygotes was assessed by comparing their ability to germinate after exposure to chloroform vapors. The bright staining of the primary wall with aniline blue, specific for  $\beta$ -1,3 polysaccharides, suggested the presence of callose. This was further supported by the adverse effects of glucanase on zygote development. After mating, declining levels of intact zygotes were found as their maturation continued, and dead immature zygotes accumulated in the treated cultures. Twelve days after mating, when the zygotes were plated for germination, fully mature zygotes were identified in only the lowest of the six enzyme concentrations. In addition, germinating zygotes from the treated cultures showed increased sensitivity to killing by chloroform vapors relative to untreated zygotes. These results suggest that callose is a key component in the primary zygote wall, and that its degradation negatively affects zygote maturation. Electron microscopy will be used to help determine whether structural defects in the primary wall occur as a result of glucanase treatment, and whether such defects affect secondary zygospore wall assembly.

## 10

### PHYLOGENY AND CLASSIFICATION OF REEF-BUILDING CORALLINE ALGAE (CORALLINALES, RHODOPHYTA)

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The Corallinales includes ca. 40 genera of calcified red seaweeds. Species are of two distinct morphotypes; those that possess genicula (uncalcified nodes) and those that lack genicula. Most nongeniculate species take the form of crusts. The presence (or absence) of genicula, secondary pit connections, and tetrasporangial conceptacle features have traditionally been used as key characters for delimiting coralline subfamilies. In this study, nuclear encoded 18S and 26S rRNA gene sequences were determined and used to reexamine relationships among coralline taxa. Separate and combined phylogenetic analyses of these data yielded similar trees in which four major lineages are resolved. *Heydrichia* and *Sporolithon* (Sporolithaceae) are positioned at the base of the tree and appear to be distantly related to other species examined. Within the Corallinaceae, the nongeniculate Melobesioideae is resolved as a monophyletic group. All members of this subfamily produce multiporate tetrasporangial conceptacles. The Corallinoideae, which are characterized by unizonate genicula, are resolved as sister to a clade containing species placed in the Lithophylloideae, Mastophoroideae and Metagoniolithoideae. The molecular data indicate that geniculate and nongeniculate species characterized

by the presence of secondary pit connections are closely related. For example, both data sets robustly support a sister taxon relationship between *Amphiroa* and *Titanoderma*. Our results indicate that: 1) all taxa in which secondary pit connections are present should be referred to the Lithophylloideae and, 2) genicula are nonhomologous structures that are independently derived in *Amphiroa*, *Lithothrix*, *Metagoniolithon* and the last common ancestor of the Corallinoideae.

## 11

### MOLECULAR SYSTEMATICS OF THE XANTHOPHYCEAE

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Species belonging to the Xanthophyceae have traditionally been classified in six (or seven) orders corresponding to amoeboid, coccoid, filamentous, monadoid, palmelloid and siphonous lines of descent. We have determined plastid *rbcL* and nuclear 18S rRNA gene sequences for selected coccoid, filamentous and siphonous species. Phylogenetic trees inferred from these data indicate that the orders Mischococcales (coccoid) and Tribonematales (filamentous) are not monophyletic. Instead, our results indicate that coccoid and filamentous life forms have arisen independently in different xanthophyte lineages. Some coccoid and filamentous species are resolved as close allies. For example, in our analyses coccoid and filamentous species possessing bipartite cell walls (e.g., *Bumilleria*, *Ophiocytium*, *Tribonema*, *Xanthonema*) form a distinct and previously unrecognized lineage within the class. The phylogenetic positions of *Asterosiphon*, *Botrydium* and *Vaucheria* are not robustly resolved, but our data suggest that these siphonous xanthophytes probably do not form a monophyletic group. Our results imply that *Vaucheria* is only distantly related to other xanthophytes we have examined. Nucleotide sequence divergence values among *Vaucheria* species were found to be equal to, or greater than, those observed between other xanthophyte species that are classified in different families or orders. Furthermore, our trees do not always support the infrageneric system of classification for *Vaucheria* species, which is based primarily upon antheridial features.

## 12

### CELL CYCLE REGULATORS IN THE FLORIDA RED TIDE DINOFLAGELLATE, *GYMNODINIUM BREVE*

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The diel cycle is a key regulator of the cell cycle in many dinoflagellates, and may play a rate limiting role in bloom formation. Diel phasing of the cell cycle in the Florida red tide dinoflagellate, *Gymnodinium breve* Davis was previously described in our laboratory. In cultures grown on a 16:8 light:dark cycle, S-phase began 6–8 h into the light phase, and mitosis followed 12–14 h later. The dark/light “dawn” transition was found to provide the diel cue that serves to entrain the *G. breve* cell cycle. However the cell cycle mechanisms and regulators acted upon by this cue are poorly understood in dinoflagellates. The cell cycle regulatory complex, CDK1-cyclinB, is therefore currently being investigated. Cyclin dependent kinase (CDK) was first identified in *G. breve* using two approaches: (1) identification of a 34 kDa protein immunoreactive to an antibody raised against a conserved amino acid sequence unique to the CDK protein family (PSTAIR) and (2) inhibition of the cell cycle by olomoucine, a selective CDK inhibitor. Several approaches are currently being employed in order to describe its partner, cyclin B: (1) PCR on genomic DNA with primers deduced from known cyclin box sequences, (2) *G. breve* expression library screening with an antibody raised against the fission yeast cyclin B (3) western blot analysis on whole protein extracts and cyclin B immunoprecipitated proteins. Current work focuses on the differential expression of the cyclin B homologue in *G. breve* during its cell cycle and its relation to diel cycle control.

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#### PHYTOFLAGELLATES OF THE SALTON SEA: CRYPTOPHYCEAE

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The species composition of phytoflagellates in the Salton Sea has recently been the subject of intense investigation as part of an analysis of the Salton Sea ecosystem. The Salton Sea, an inland sea occupying 980 km<sup>2</sup> in southern California, has become a major stopping point for migratory birds along the Pacific Flyway. The increasing salinity of the sea, currently at 44 gm L<sup>-1</sup>, and its eutrophic condition (average depth is nine meters, with a high nutrient load contributed by agricultural drainage from the surrounding farmlands) have contributed to a stressed ecosystem. Massive fish kills and bird kills, including such endangered birds as the brown pelican, have become a recurring problem. Although previous investigations have noted the presence of at least two phytoflagel-

lates implicated in fish mortality, little attention has been paid to the identities of the smaller flagellates observed growing in the sea and their possible contribution to the fish and bird population mortality. Using freshly collected field samples as well as enrichment culture techniques, we report the occurrence of several genera of cryptomonads in the Salton Sea, including representatives from the genera *Chroomonas*, *Hemiselmis*, *Leucocryptos*, *Plagioselmis*, *Storeatula* and *Teleaulax*.

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#### STRUCTURE, COMPOSITION AND BIOGENESIS OF PRASINOPHYTE SCALES

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The cell body and flagellar surfaces of prasinophytes are covered by non-mineralized scales. Scales consist mainly of acidic polysaccharides containing large amounts of 2-keto sugar acids. Glycoproteins are minor components and probably mainly involved in mediating scale-subunit and scale-membrane interactions. In thecate prasinophytes the cell body scales coalesce to form a rigid cell wall, generally known as a theca. We have studied the polysaccharides and glycoproteins of the thecate prasinophytes *Tetraselmis striata* and *Scherffelia dubia* over the last years. New results regarding the structure of carbohydrates and proteins will be presented.

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#### PHYLOGENY OF ATLANTIC SPECIES OF GRACILARIACEAE (RHODOPHYTA) BASED ON SMALL SUBUNIT RIBOSOMAL GENE SEQUENCING

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The complete small subunit ribosomal nuclear gene (ssu rDNA) sequence was determined for nine species of *Gracilaria* and one species of *Gracilariopsis* which are common on the American Atlantic waters. The sequences were aligned using the secondary structure as reference, including the published sequences of nine other species of Gracilariaceae. A matrix of 1736 sites was constructed with a proportion of 91% invariable sites and very few assumed indels events. All the inferred trees show three main lineages: 1) the strongly divergent lineages of *Gracilariopsis*; 2) the austral genera *Curdiea*/*Melanthalia*; and 3) the lineage of *Gracilaria sensu stricto*. The later encompasses the following groups: 1) *Gracilaria chilensis* from the Pacific

ocean; 2) a group of cylindrical tropical species with "henriquesiana" spermatangial type; 3) a group of warm temperate cylindrical species with "verrucosa" spermatangial type; and 4) a group of flattened tropical species with mainly "textorii" spermatangial type. The relationship of a species described as *Gracilaria pauciramosa* from Venezuela was not unequivocally solved. The inferred phylogenetic groups are congruent with morphology and quality of agar.

## 16

### ARE SPERM LIMITING IN THE SEA?

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The reproductive success of marine species with external fertilization depends on environmental conditions during gamete release. There is special interest presently in whether water motion causes sperm limitation under natural conditions. We investigated gamete release of *Fucus vesiculosus* from an exposed shore to ascertain: 1) when gametes are released during the tidal cycle, 2) when fertilization occurs, and 3) what the natural sperm:egg ratios are. Water samples were collected and concentrated over five minutes every half hour off Pemaquid Point, ME from three replicate sites within each of two locations using a pump-filter device. Immunofluorescence microscopy revealed that gamete release occurred only on the two calmest spring tides. Sperm became present in the water column at the same time as oogonia ( $30 \text{ min}^{-1} \text{ h}$  prior to high tide [HT]) and reached peak concentration at exactly HT. The sperm:egg ratio was 76:1 on 8 Oct 1999 and 21:1 on 8 Nov 1999 at exactly 30 min prior to HT and dropped sharply after HT. Gametes continued to be collected for several hours after HT but analysis of pronuclear position in aceto-iron-hematoxylin stained eggs revealed that all fertilization occurred at approximately HT. We modelled the total number of days when reproduction was possible using these results and wind and wave data from the National Data Buoy Center. Our research provides evidence that gamete release by *F. vesiculosus* occurs at slack HT on calm days and that sperm are not a limiting factor in fertilization for this species.

## 17

### PATTERNS OF INTERSPECIFIC INTERACTIONS IN THE ULVA-DOMINATED INTERTIDAL COMMUNITY IN A SOUTHERN COAST OF KOREA

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In the southern coast of Korea, rocky intertidal zones where green tide commonly occurs are dominated by

*Ulva pertusa*, red algal turf (a mixed stand of *Gigartina intermedia* and *Gigartina teedii*) and species of *Enteromorpha* with highly opportunistic occurrence. To investigate their interspecific interactions, a field experiment was carried out using press effect of one species removal from permanent plots (20 x 20cm) set up on two different tidal heights. Mechanisms of interaction were also attempted using artificial plants to test the possible effects of shading, scouring, allelopathy of *U. pertusa* on the turf algae. The turf-forming red algae lowered the abundance of *U. pertusa* presumably by inhibiting the recruitment of *U. pertusa*; this effect was consistent along the tidal height. However, the greater abundance of *Enteromorpha* was observed in the presence of turf in the upper zone, indicating a positive effect of turf on *Enteromorpha*. Once *U. pertusa* was successfully recruited and grown to the adult plant, it inhibited the growth of turf by shading, which was effective both in winter and summer regardless of desiccation stress. No scouring and allelopathic effects of *U. pertusa* on the turf were detected. When an open substrate was provided, *Enteromorpha* colonized the space faster than any other species in the upper zone, but the turf was the fastest one followed by *U. pertusa* and *Enteromorpha* in the lower zone. Results indicated that patterns of interaction represented a complex network with no ultimate winner and the outcomes of interaction varied over time and space.

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### USING RDNA GENES TO UNDERSTAND THE ORIGIN AND EVOLUTION OF SPLICEOSOMAL AND GROUP I INTRONS

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Ribosomal DNA genes in lichen algae and lichen fungi are astonishingly rich in spliceosomal and group I introns. We use phylogenetic, secondary structure, and biochemical analyses to understand the evolution of these introns. Despite the widespread distribution of spliceosomal introns in nuclear pre-mRNA genes, their general mechanism of origin remains an open question because few proven cases of recent and pervasive intron origin have been documented. The lichen introns are valuable in this respect because they are undoubtedly of a "recent" origin and limited to the Euascomycetes. Our analyses suggest that rDNA spliceosomal introns have arisen through aberrant reverse-splicing (in trans) of free

pre-mRNA introns into rRNAs. We propose that the spliceosome itself (and not an external agent; e.g. transposable elements, group II introns) has given rise to the introns. The rDNA introns are found most often between the flanking sequence G (78%) - intron-G (72%), and their clustered positions on secondary structures suggest that particular rRNA regions are preferred sites (i.e., proto-splice sites) for insertion. Mapping of intron positions on the newly available tertiary structures show that they are found most often in exposed regions of the ribosomes. This again is consistent with an intron origin through reverse-splicing. Remarkably, the distribution and phylogenetic relationships of most group I introns in nuclear rDNA genes are also consistent with a reverse-splicing origin. These data underline the value of lichens as a model system for understanding intron origin and stress the importance of RNA-level processes in the spread of these sequences in nuclear coding regions.

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#### **INTRA-CELLULAR AND EXTRA-CELLULAR TOXINS IN THE RED TIDE DINOFLAGELLATE, *GYMNODINIUM BREVE***

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The marine dinoflagellate, *Gymnodinium breve* (Davis), produces several neurotoxins that cause neurotoxic shellfish poisoning (nsf), massive fish kills and respiratory irritation in marine mammals and humans. The common method for discerning toxic levels of *G. breve* for public health advisories is enumeration of live cells in a given water mass. In this study, laboratory cultures, as well as natural blooms, were added to a stirred ultra-filtration cell concentrator to separate viable cells containing intra-cell toxins from ambient water containing extra-cell toxins. Methods were validated using various mixtures of lysed and whole *G. breve* laboratory culture. Extractions and recovery of brevetoxins were done using a C-18 bonded-phase glass fiber extraction disc eluted with methanol. Total PbTx toxin concentrations were quantified by HPLC/UV using a C-18 column and an 85:15 methanol:water (1 ml min<sup>-1</sup>) isocratic elution at 215 nm. This method of separation and extraction was subsequently applied to water samples collected during natural blooms along two different areas of the Florida Gulf coast. The results indicated that early stages of *G. breve* blooms contained primarily intra-cell toxins with extra-cell toxins increasing as the bloom progressed, even though very few viable *G. breve* cells were present. This suggests that enumeration of cells alone may be insufficient and additional toxin quantitation is necessary.

20

#### **A RE-EVALUATION OF CORALLINE RED ALGAL TAXONOMY USING ULTRA-STRUCTURAL INFORMATION**

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Two competing subfamily classification schemes for the red algal family Corallinaceae are in general agreement with respect to the taxonomic value of cell connection type (cell fusions versus secondary pit connections), but differ over the relative weight given to the character of genicular presence/absence. Consequently, Corallinoideae, Melobesioideae and Metagoniolithoideae are recognized as distinct subfamilies. One scheme subsumes the geniculate Amphiroideae into the nongeniculate Lithophylloideae (based on the common presence of secondary pit connections) and the nongeniculate Mastophoroideae into the geniculate Corallinoideae (based on the common presence of cell fusions); the other maintains these subfamilies as four separate lineages. Small subunit rRNA gene sequence data confirms the integrity of Corallinoideae and Melobesioideae and fully supports a revised classification subsuming Amphiroideae into Lithophylloideae. Surprisingly, Mastophoroideae and Metagoniolithoideae (cell fusions) have a closer affinity to the emended Lithophylloideae (secondary pit connections) than with Corallinoideae (cell fusions). These preliminary results cast doubt on the value of using cell connections as a primal character. From ultrastructural investigations of 28 corallinoidean genera, including seven mastophoroidean genera and two lithophylloidian genera, it appears that the presence/absence of an electron-dense material (EDM) surrounding nuclei during sporogenesis is a more reliable indicator of subfamily affiliation than cellular connections. Our findings support the revised classification of Lithophylloideae and suggests closer affinity to Lithophylloideae (secondary pit connections, no EDM) than to Corallinoideae (cell fusions, EDM). However, EDM data indicates that Mastophoroideae may be polyphyletic as three genera have EDM and four do not.

21

#### **PORPHYRA SUBORBICULATA, PORPHYRA CAROLINENSIS AND PORPHYRA LILLIPUTIANA - THREE NAMES FOR ONE SMALL PORPHYRA**

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The genus *Porphyra* is ancient, successful, and morphologically simple. Its members provide a particular challenge to systematists who must decide whether shared features are a result of homoplasy, or reflect recent common ancestry. Three species of diminutive *Porphyra* with widespread geographic origins share many common morphological features: *P. suborbiculata* Kjellm. has been reported from the west Pacific and Indian Oceans, *P. carolinensis* Coll et J. Cox from the west Atlantic, and *P. lilliputiana* W. A. Nelson, G. A. Knight et M. W. Hawkes from New Zealand. Comparison of 18S rDNA sequence data from small *Porphyra* thalli from Mexico, Japan, New Zealand, Australia and Connecticut indicate that these three taxa in fact belong to one cosmopolitan species, which is distributed over three oceans and two hemispheres. Analysis of sequence data from introns present in the 18S rDNA and from the ITS region suggest that this distribution may be linked to human activity, and raises the question of to which geographic locality this entity is truly endemic?

## 22

### ALGAE AS USEFUL TOOLS IN CELLULOSE RESEARCH

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This presentation will review the 1976 discovery of the enzyme complex in cellulose biosynthesis in *Oocystis apiculata*. A linear terminal complex (TC) was found to be associated with a microfibril, and from other freeze fracture applications, TCs have been found in many different algal genera. In fact, the algae have the most diverse and complex TCs among all organisms. TC diversity in terms of the evolution of cellulose biogenesis will be discussed. Combining the latest information from biochemistry and molecular genetics, the multiplicity of cellulose biogenesis will be reviewed. Cellulose molecular weight, crystalline structure, and mode of glycosylation for polymer formation all indicate that cellulose biogenesis is an extremely complex process. Major questions still remain, and the enzymes for cellulose biosynthesis have yet to be crystallized and their structure elucidated; however, the wealth of new information on cellulose structure and biosynthesis from algae to vascular plants, including bacteria and tunicates, all point to a very exciting and useful area of research. The algae

have played key roles in our understanding of nature's most abundant macromolecule.

## 23

### CHARACIOCHLORIS AND CHARACIOSIPHON FORM A SISTER GROUP TO THE "DUNALIELLA" LINEAGE

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The freshwater green algal genera, *Characiochloris* and *Characiosiphon*, are distinctive taxa for which no clear consensus exists regarding phylogenetic placement. *Characiochloris* is an epiphytic unicell that exhibits multiple (>2), scattered contractile vacuoles throughout the cytoplasm of the vegetative cell. Although *Characiochloris* has been placed in the Tetrasporales by most systematists, the uncertain status of this green algal order raises questions regarding the position of *Characiochloris*. *Characiosiphon*, a coenocytic freshwater organism, has been linked to another freshwater coenocyte, *Protosiphon*. However, multiple chloroplasts, greater thallus size, and a strictly aquatic life history for *Characiosiphon* have led to comparisons with caulerpalean ulvophytes. Phylogenetic analysis of 18S and 26S rDNA data was undertaken to test hypotheses of relationship for *Characiochloris* and *Characiosiphon*. Results from molecular phylogenetic analyses strongly support a sister relationship for *Characiochloris* and *Characiosiphon*. In addition, the *Characiochloris/Characiosiphon* clade is resolved as a sister group to the "Dunaliella" lineage of the Chlamydomonadales that includes *Ascochloris*, *Chlorogonium*, *Dunaliella*, *Haematococcus*, *Protosiphon* and *Spongiocloris*. These results further support the hypothesis that the "Dunaliella" clade includes the majority of taxa that exhibit multiple contractile vacuoles. Moreover, these results support an interpretation of the *Characiosiphon* coenocyte as a modified zoosporangium.

## 24

### PSEUDOFILAMENTOUS GREEN ALGAE FORM INDEPENDENT CHLOROPHYTE AND STREPTOPHYTE LINEAGES

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The pseudofilamentous condition in green algae has been characterized as the formation of a linear array of autospores. Although it is likely that this characterization will be found to be an over-simplification, it serves as a logical starting point for a study of diversity among pseudofilamentous taxa. Therefore, given that



molecular phylogenetic analyses have revealed that coccoid, autospore-formers are found in a number of independent green algal lineages, it is reasonable to expect that pseudofilamentous taxa are likely to be found in a number of distinct lineages. In order to test this hypothesis, data from the nuclear-encoded 18S rRNA gene were collected from several pseudofilamentous, green algal genera (*Geminella*, *Gloetila*, *Hazenia*, *Interfilum*, *Microspora*, and *Sphaeroplea*) and incorporated into an 18S rRNA database of chlorobiont taxa. Results from phylogenetic analyses of these data support (1) an alliance of *Interfilum*, two *Geminella* isolates, and *Klebsormidium* within the streptophyte lineage, (2) an alliance of two *Geminella* isolates and *Microspora* as a sister group to the ulvophycean/chlorophycean/trebouxiophycean clade or as a sister group to trebouxiophycean taxa, (3) an alliance of *Hazenia* with ulotrichalean taxa, (4) of *Gloetila* with trebouxiophycean taxa and (5) an alliance of *Sphaeroplea* with chlorophycean taxa. These data confirm that the filamentous condition has evolved in a number of independent lineages. Moreover, these data further illustrate that the extent of molecular variability within the green algae is far from fully appreciated.

## 25

### THE EFFECT OF NITROGEN SOURCE ON THE GROWTH AND TOXICITY OF THREE POTENTIALLY HARMFUL DINOFLAGELLATES

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Increases in population and agriculture in coastal areas can result in increased nutrient inputs and alterations in the ratios of organic to inorganic nutrients in coastal waters. Such changes in coastal nutrient regimes can affect phytoplankton community structure by creating conditions favorable for growth and dominance of algae that were not dominant before. The effect that changes in ratios and concentrations of nutrients have on toxicity of harmful algal species is not well known. There seems to be a relationship; however, between nutrient stress and toxin production among harmful phytoplankton producing low-N toxins, e.g. Diarrhetic Shellfish Poisoning (DSP) toxins. Even less is known about the relationship between organic nutrient uptake and toxin production. Benthic species and species in coastal areas are probably exposed to greater fluxes of dissolved organic nitrogen

(DON). In this study, benthic and planktonic species of *Prorocentrum* were grown on L1 media with the sole N-source varying among treatments as nitrate, ammonium, urea, L-glutamic acid, and high molecular weight natural DON. An ELISA specific to the DSP toxins, okadaic acid and 35-methylokadaic acid, was used to determine toxin production by each species when grown on the different N sources. Preliminary results indicate that some organic forms of N support growth as well as inorganic forms for *Prorocentrum minimum*, *P. mexicanum*, and *P. hoffmannianum*.

## 26

### CULTIVATION STUDIES OF *GIGARTINA SKOTTSBERGII* IN SOUTHERN CHILE

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In Chile, the demand for carrageenophytic algae has increased significantly in recent years. The preferred species is *Gigartina skottsbergii*, with landings of 26,181 tons in 1998. To avoid a possible overexploitation the development of cultivation technologies is needed. In this study we present a critical view of published and new information to propose the best culture strategy for this species. The results indicate that viable spores are seasonally available only during winter, with tetraspore germination rates of ca. 40%. Germination of carpospores is lower than 20%. Growth in tanks can be as high as 1% day<sup>-1</sup> and high nutrient concentrations (N and P) must be added. *Gigartina* also present a high and unique regeneration capacity. Field experiments indicate that *Gigartina* can be cultured in rope systems, where the inoculum can be tissue fragments. These results indicate that vegetative propagation techniques can be used for the massive culture of *Gigartina*, but selection of a high growing strain remains as one of the future challenges.

## 27

### MONITORING THE PERSISTENCE OF GIANT KELP AROUND SANTA CATALINA ISLAND USING A GEOGRAPHIC INFORMATION SYSTEM

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Geographic information systems (GIS) facilitate monitoring and analysis of population distributions at spatial and temporal scales differing from those em-

ployed in conventional field monitoring. This study utilizes a GIS-based gap analysis of a network of marine reserves around Santa Catalina Island relative to the regional ecology, disturbance regime, and persistence of giant kelp (*Macrocystis pyrifera*), a keystone species in the nearshore, marine environment. Catalina's orientation and greatly-dissected coastline create diverse microhabitats with respect to storm exposure, temperature, light regime and topographic factors. GIS overlay methods applied to multi-temporal kelp distribution maps generated a model representing the spatial "persistence" of kelp. Correlations between the kelp's geographic distribution and persistence, the disturbance regime and physical variables conferring resistance to or recovery from it were drawn. This analysis identified regions of persistent kelp under disturbance regimes markedly different from those in the existing reserves, suggesting the designation of additional reserves in unprotected areas is ecologically warranted.

## 28

#### **EFFECT OF TEMPERATURE AND DESICCATION ON THE PHOTOSYNTHETIC PERFORMANCE OF *PORPHYRA PERFORATA***

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*Porphyra perforata* is a common seaweed inhabiting the upper intertidal zone, and as a consequence it experiences great fluctuations in tissue temperature and desiccation. The objective of this work was to evaluate the effect of ambient temperature and the tissue desiccation status on the photosynthetic performance of *P. perforata*. Photosynthetic performance was evaluated polarographically after the temperature or desiccation treatments. Maximum photosynthesis ( $P_{\max}$ ) occurred between 25 and 30° C and decreased at higher and lower temperatures, however, no significant differences were observed in the initial slope of photosynthesis ( $\alpha$ ) from 10 to 30° C. This suggests that the photosynthetic efficiency of this species does not decrease as a result of fluctuating temperatures during tidal emergence/submergence.  $P_{\max}$  and  $\alpha$  were relatively constant in tissue of *P. perforata* with 5 to 100% relative water content. This also suggests that natural desiccation rates during low tides do not decrease photosynthetic rates in this species. Variations in the synthesis of specific proteins as a result of fluctuations in temperature and relative water content in the tissue of *P. perforata* are being studied.

## 29

#### **EFFECT OF LIGHT QUALITY ON POLYSACCHARIDE YIELD AND COMPOSITION OF TWO RED ALGAE**

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Two species of agarophytes, *Gelidium sesquipedale* (Clem.) Born. et Thur. and *Gracilaria tenuistipitata* var. *liui* (Zhang & Xia), have been cultured in chemostat systems under different light qualities to study the response of growth and polysaccharide yield and composition to certain wavelengths. The yield of galactans, methoxyl groups and sulphate content in the agar as well as the starch concentration of these species are controlled by light quality in a different way, presumably because of their patterns of growth. The polysaccharide characteristics will be discussed in relation to the growth and metabolism of those algae.

## 30

#### **DO PHYSICAL FACTORS REGULATE PHYTOPLANKTON DISTRIBUTION PATTERNS IN LARGE, SHALLOW LAKES?**

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Factors that regulate phytoplankton dynamics in shallow, productive lakes are poorly understood, due to their predisposition for frequent algal blooms and sediment resuspension events. In Lake Apopka, greatest phytoplankton biomass reflects wind-induced resuspension of algae (meroplankton) that exists on the aphotic lake bottom in a layer approximately 5 cm thick; this assemblage is dominated by diatoms (>60% of total biomass) that can occur in resting stages. Once exposed to moderate light, meroplankton are capable of growth and photosynthetic rates comparable with surface populations. In Lake Okeechobee, remote sensing was used to assess the basin-wide distribution of suspended particles. Satellite reflectance values agreed well with *in situ* particle densities at 20 in-lake stations (average  $r^2$ ; LANDSAT = 0.81, AVHRR = 0.53), and maps of algal blooms ( $r^2 = 0.79$ ,  $p \leq 0.01$ ). The greatest chlorophyll concentrations

occurred in the vicinity of tributary nutrient inputs at the lake's perimeter, while turbidity increased towards the center of the lake reflecting predominant water circulation patterns. These results underscore the importance of physical-biological interactions in lakes.

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### **FRESHWATER DINOFLAGELLATES OF BELIZE, C.A.**

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Freshwater dinoflagellates have not previously been reported from Belize, although there has been extensive work with marine dinoflagellates and some work with other freshwater groups. Freshwater dinoflagellates are more frequently observed in standing water and none have been observed in the several streams and rivers sampled since 1990. The goal in 1998 was to examine water samples from small ponds within hours of collection to improve the chance of observing swimming dinoflagellates. A plankton net was used and whole water samples also were collected. A small brown water pond on a peninsula and 30m from the Caribbean yielded a bloom of *Thompsodinium intermedium*. Dinoflagellates, including *Peridinium centenniale*, *Katodinium* sp., and *Peridinium* sp. in the *Umbonatum* Group, were observed within "Crocodile pond" and "Lily pond" on the mainland.

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### **SOME PHYLOGENETIC RELATIONSHIPS WITHIN THE OSCILLATORIALES (CYANOBACTERIA) CLADE USING 16S rDNA GENE SEQUENCE DATA**

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An approximately 1400 base pair region of the 16S rDNA gene was sequenced from taxa within the Oscillatoriales in order to assess phylogenetic relationships. Ten previously unsequenced strains were obtained from the University of Toronto Culture Collection. New sequence data were combined with previously published sequences from a wide representation of cyanobacteria including all currently available, complete Oscillatorialian taxa. Trees constructed using parsimony, distance, and maximum likelihood methods were similar in topology, although a few taxa were variable in their placement depending on the phylogenetic method employed. Newly sequenced taxa of the genera *Phormidium*, *Oscillatoria*, and *Lyngbya* did not form monophyletic clades based on traditional generic designations. Two *Lyngbya* strains (UTCC296 and 313) and *Phormidium subfuscum* (UTCC474) formed a well supported monophyletic clade, but the affinity

of this clade with other groups was uncertain due to lack of bootstrap support. *Oscillatoria* sp. (UTCC393) was closely related to the previously sequenced *Oscillatoria limnetica* and likewise, *Phormidium molle* (UTCC77) and *Phormidium tenue* (UTCC473) were placed in a well supported clade with other Oscillatoriales. The other four taxa were variously placed in the trees and their phylogenetic positions could not be determined with certainty.

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### **ULTRASTRUCTURE AND CYTOCHEMISTRY OF SPERMATANGIAL DEVELOPMENT AND FERTILIZATION IN *AGLAOTHAMNION OOSUMIENSE* (CERAMIACEAE, RHODOPHYTA)**

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Spermatial development and fertilization processes were investigated by electron microscopy in *Aglaothamnion oosumiense* Itono. The spermatium is composed of two parts, an ovoidal head and two appendages projecting from each distal end. The appendages originate from spermatangial vesicles (SVs) and follow a developmental sequence beginning as amorphous material and ending as a fully-formed fibrous structure compressed within the SVs. SV formation is due to contributions initially from endoplasmic reticulum and later from dictyosome-derived vesicles. Chemical differentiation of the spermatial wall occurs early in its development. Calcofluor white does not label spermatial walls, indicating an absence of cellulose polysaccharides, which are abundant in vegetative cell walls. Liberated spermatia had a prophase arrested nucleus with a pair of polar rings. The cytoplasm of the trichogyne was connected with that of spermatia at the fertilization canal. The cytoplasm of the trichogyne was empty when the nuclear fusion between spermatium and carpegonium occurred.

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### **COMPARISON OF GROWTH AND REPRODUCTION BETWEEN TWO POPULATIONS OF *SARGASSUM SILIQUASTRUM* IN PING CHAU, HONG KONG**

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In Ping Chau Island of Hong Kong, two populations of *Sargassum siliquastrum* are present. One is in the shallow water of about 1 to 3 m Chart Datum (CD), and the other one in deeper water of 5 to 10 m CD. These two populations are separated by an extensive

sand patch. Individuals of the "shallow" water population increased their size from a mean length of  $8.3 \pm 3.6$  (SD) cm in Aug 1998 to a maximum of  $48.2 \pm 29.9$  cm in early Jan 1999 before they started to die back. In the following year, they attained a minimum of  $6.1 \pm 3.8$  cm in May 1999 and a maximum of  $56.1 \pm 23.6$  cm in Dec 1999. Their reproductive period lasted for two to three months from Jan to Feb 1999, and again from Nov 1999 to Jan 2000. The "deep" water individuals increased their size to a maximum of  $123 \pm 50.8$  cm at the end of Jan 1999 and started to die back in Feb, 1999. They again reached their maximum mean length in Jan 2000. Their reproductive period lasted for five to six months from Sept 1998 to Feb 1999 and again from Sept 1999 to Jan 2000. The "deep" water individuals tended to be longer in size and they attained their maximum growth a month later than the "shallow" water individuals. Their reproductive season tended to start earlier and lasted longer than those in the "shallow" water. These differences in the phenology of the two populations may be related to the temperature differences (up to  $5^\circ$  C difference in summer) between the two depths. *Sargassum siliquastrum* is likely to be a cold adapted species such that warmer temperature in the shallow water has compressed and shortened their rapid growth and reproductive period to within the few colder months in fall and winter.

## 35

**SEXUAL REPRODUCTION AND GENETIC VARIATION IN THE SUBAERIAL ALGA *CEPHALEUROS VIRESCENS* (ULVOPHYCEAE, CHLOROPHYTA)**

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*Cephaleuros virescens* is a pantropical subaerial green alga with no known long-range dispersal mechanisms. Sexual reproduction is relatively rare and may involve intragametangial fusion of identical, mitotically produced gametes. This situation may be a consequence of adaptation to the subaerial habitat. Genetic variation among populations of *C. virescens* may be very low and might be positively correlated to the distance (hence, time) separating populations. Thus, assessing the global biogeography of *C. virescens* requires analysis of what might be low levels of variation. Because *C. virescens* occurs on literally hundreds of different host species, the question of host-races must also be considered. Preliminary analysis of local populations of *C. virescens*, originally obtained as field collections from three different host species and subsequently raised in culture, is the first step in addressing the biogeography

of this alga. We are using the AFLP plant mapping protocol by PE Applied Biosystems to detect genetic variability in the three isolates of *C. virescens*. AFLP is a PCR-based DNA fingerprinting technique that detects the presence or absence of restriction fragments rather than fragment length differences. Because the number of restriction fragments that can be detected with the AFLP technique is "virtually unlimited," it is a very powerful tool for assessing the degree of relatedness or variability among cultivars or isolates. AFLP techniques have been used successfully to distinguish morphologically identical bacteria, determine relatedness among soybean accessions, reveal genetic variability within bee samples, and identify fall armyworm strains and hybrids.

## 36

**COLD ACCLIMATION OF TWO STRAINS OF THE CYANOBACTERIUM, *SPIRULINA PLATENSIS***

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The changes in the antioxidant enzymes and photosynthetic activities during cold acclimation in two strains of the cyanobacterium, *Spirulina platensis*, marked as Kenya and M2, were investigated. Cells were cultured at  $33^\circ$  C then transferred to either  $20^\circ$  C or  $15^\circ$  C. Growth rate of both strains ceased when the temperature shifted from  $33$  to  $20^\circ$  C and resumed to a slower rate after the first 24 h. Similarly when the temperature was shifted from  $33$  to  $15^\circ$  C, a complete cessation of growth took place but this time the growth resumed only after 5 to 6 days. The activities of ascorbate peroxidase/ peroxidases were immediately decreased to about 40–60% in Kenya and 20–40% in M2 when the temperature was shifted to 20 or  $15^\circ$  C and remained about the same level during further cold cultivation. The activity of glutathione reductase was not modified immediately after the transfer to 20 or  $15^\circ$  C; however, it tended to increase gradually in M2 during cultivation at  $15^\circ$  C. The changes in the maximal efficiency of PS<sub>II</sub> photochemistry (Fv/Fm) were followed in order to study the effect of low temperature acclimation on the photosynthetic apparatus of the two strains.

## 37

**ATLANTIC KELP SPECIES *LAMINARIA LONGICRURIS* AND *L. SACCHARINA* (LAMINARIALES) ARE CONSPECIFIC**

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The identification of two Atlantic kelp species, *Laminaria longicuris* and *L. saccharina*, has been controversial. In order to know if these two species are conspecific, plastid-encoded RuBisCo spacer and nuclear rDNA ITS sequences were analyzed from twelve individuals of *L. longicuris*, five of *L. saccharina*, from Connecticut and Nova Scotia. Four individuals of *L. digitata* from the above coasts were also analyzed as reference in this study. All RuBisCo spacer sequences from *L. longicuris* and *L. saccharina* were exactly identical except for one individual with three different nucleotides. Zero to five different nucleotides of ITS sequences, including four polymorphic sites, were found in two species. However, their RuBisCo spacer and ITS sequences are quite different from those of *L. digitata*. These results strongly suggest that *L. saccharina* (L.) Lamour. 1813 is conspecific with *L. longicuris* De La Pylaie 1824 and the different local populations, despite various morphotypes, may be genetically very similar.

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#### TAXONOMY OF CERAMIUM (CERAMIAEAE, RHODOPHYTA) FROM OREGON

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During a recent survey of marine red algae in Oregon, U.S.A., 6 of the 7 species of *Ceramium* reported from this state were collected in the intertidal zone. These included *Ceramium californicum* J. Agardh, *C. cimbriicum* H. Petersen in Rosenvinge, *C. codicola* J. Agardh, *C. gardneri* Kylin, *C. kondoi* Yendo, and *C. pacificum* (Collins) Kylin. Detailed descriptions and illustrations of vegetative and reproductive development in *C. californicum*, *C. codicola*, and *C. pacificum* are presented for the first time in this paper. Descriptions are also given for *C. cimbriicum* and *C. kondoi*, species previously only reported from elsewhere in the northwestern Pacific. These latter species were common on floating docks, sites where introduced species frequently occur. *Ceramium eatonianum* and *C. washingtoniensis*, the other 2 species known from Oregon, were found to belong to other genera or were not collected during this study.

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#### POLYPHOSPHATE AND SILICEOUS GRANULES IN THE GAMETOPHYTES OF THE RED ALGA PORPHYRA PURPUREA (BANGIOPHYCEAE)

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Variations of the different phosphorylated fractions in tissues of starved and fresh plants of *Porphyra purpurea*, a representative of the Bangiophyceae, over a 72 hour incubation period in pulse-enriched seawater (15 μM phosphorus and 25 μM nitrogen) were analyzed and compared to those in *Chondrus crispus*, a representative of the Florideophyceae, considered the more advanced of the two classes of the Rhodophyta. Differences point towards *P. purpurea* being a much more metabolically active phosphorus "pump" than *C. crispus*, with a higher phosphorus turnover rate, in which the orthophosphate fraction is predominant and acid-soluble and acid-insoluble polyphosphates are not as significant storage pools. Confirmation of the presence of acid-insoluble polyphosphates, detected by chemical analyses, in the form of cytoplasmic granules was obtained by transmission electron microscopy and energy dispersive X-ray microanalysis. The granules in *P. purpurea* were, however, much smaller (20 to 110 nm in diameter) than those in *C. crispus* (around 1 μm, but some larger than 2 μm in diameter). Larger granules (290 to 310 nm in diameter) were also observed. Their surface was more uniformly electron-opaque without the reticulated or globular appearance of typical polyphosphate granules. Energy dispersive X-ray microanalysis demonstrated that they were siliceous granules. To our knowledge, this is the first report in algae of such structures whose occurrence and metabolic role remain enigmatic.

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#### MOLECULAR AND GENETIC ANALYSIS OF A RETROGRADE SIGNALING PATHWAY FROM THE PLASTID TO THE NUCLEUS

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Chloroplast development requires coordinate nuclear and chloroplast gene expression. A putative signal from the chloroplast couples the transcription of certain nuclear genes encoding photosynthesis-related proteins with chloroplast function. We have identified five *Arabidopsis* nuclear genes (*GUN1-GUN5*) necessary for coupling the expression of some nuclear genes to the functional state of the chloroplast. Homozygous recessive *gun* mutations allow nuclear gene expression in the absence of chloroplast development. *GUN1* mutants have no visible phenotype in white

light, but *GUN1* mutations interfere with the switch from heterotrophic to photosynthetic growth in young seedlings. *GUN2-5* are pale. Double mutant studies suggest that *gun1* affects a separate pathway from *GUN2*, 3, 4, and 5. *GUN2* and *GUN3* are allelic to the known photomorphogenetic mutants, *hy1* and *hy2*, involved in phytochromobilin biosynthesis downstream from heme. *GUN5* encodes the ChlH subunit of Mg-chelatase, and *GUN4* encodes a novel chloroplast protein that is essential for chlorophyll accumulation. *GUN4* does not appear to be required for the synthesis of protochlorophyllide from 5-aminolevulinic acid, which suggests that *GUN4* might be required for the early steps and/or the late steps of chlorophyll synthesis or another process that is required for chlorophyll accumulation. Our data suggest that certain perturbations of the tetrapyrrole biosynthetic pathway generate a signal from chloroplasts that causes transcriptional repression of nuclear genes encoding plastid-localized proteins. The precise nature of this signal and the mechanism by which this signal is transduced to the nucleus is under investigation.

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**BUILDING A SPECIES PHYLOGENY FOR THE GENUS *COLEOCHAETE* (COLEOCHAETALES) USING THE GENES *RBCL* AND *ATPB***

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The freshwater green alga *Coleochaete* Breb. (Coleochaetaceae; Coleochaetales) is a key streptophyte genus and is important to the understanding of the evolutionary origin of embryophytes (land plants). To date only a few species have been available from public culture collections. To facilitate research on this genus we have isolated 17 previously uncultured species of *Coleochaete* from material collected in the United States, Puerto Rico, and the Dominican Republic. Sequences for the genes *rbcL* and *atpB* were determined for these new isolates of *Coleochaete* (and for existing cultures) and combined with sequences from representative other streptophytes. Phylogenetic analyses indicate that *Coleochaete*, along with *Chaetosphaeridium* and *Chara*, are closely related to embryophytes and constitute a 'higher streptophyte' clade. At least four well-supported lineages exist within *Coleochaete*. Characteristic growth forms have been identified for these four lineages, with important characters including aspects of thallus establishment, thallus habit, zygote development and hair sheath position. These data provide an improved understanding of species diversity and character evolution in the genus *Coleochaete*, and facilitate examination of hypotheses concerning character evolution in the streptophytes.

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**PHYLOGENETIC RELATIONSHIP OF *COLEOCHAETE* AND *CHAETOSPHAERIDIUM* (COLEOCHAETALES) BASED ON THE CHLOROPLAST GENES *RBCL* AND *ATPB***

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The freshwater green algal genera *Coleochaete* and *Chaetosphaeridium* form the order Coleochaetales sensu Mattox and Stewart (Charophyceae). To test the monophyly of this order, a data set was compiled consisting of the chloroplast genes *rbcL* and *atpB* from nine species of *Coleochaete*, six strains of *Chaetosphaeridium*, and other representative green algae and embryophytes. Phylogenetic analyses of these data indicate that *Coleochaete* and *Chaetosphaeridium* form a monophyletic group that diverged late in basal streptophyte evolution. By contrast, published analyses of nuclear encoded small subunit ribosomal DNA (rDNA) data for similar taxa do not support a monophyletic Coleochaetales. These analyses suggest *Chaetosphaeridium* is an early branching lineage within Streptophyta and/or that *Chaetosphaeridium* forms a lineage with the unicellular flagellate *Mesostigma* (Mesostigmatophyceae). A close relationship of *Chaetosphaeridium* and *Mesostigma* is not supported by the *rbcL* and *atpB* data. Reexamination of morphological characters suggests a monophyletic Coleochaetales is supported by several characters that include branching filamentous habit, unicellular apical growth, sheathed hairs, and rotating plastids.

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**HABITAT HETEROGENITY ON TROPICAL ROCKY SHORES: A SEAWEED STUDY IN SOUTHERN BRAZIL**

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The main concepts in coastal community ecology have been proposed to explain relatively plain substrate, specially at temperate zones. We show that, on a irregular tropical substratum (a complex system of quarry stones with diverse shapes and sizes), the classical community zonation scheme is only applicable if we simplify the tridimensional substrate arrangement to a single dimensioned universe: the topographic level. Thus, the continuous spatial distribution implic-

ited in “zones” notions is nonexistent. Our study was conducted on a rocky shore at Ubatuba (São Paulo State, Brazil) during one year. Percentual coverage was estimated using the quadrat method located by random coordinates. The topographic level was determined for each sampling element. Analysis of spatial splitting revealed large-scale stratification of the shore into dominiums related to topographical level: 1) Upper Dominium (represented by *Chthamalus* sp. and *Tetraclita spalactifera*); 2) Transition Level (composed of *Gymnogongrus griffithsiae*, *Gelidium aff. pusillum* and often *Centroceras clavulatum*);. and 3) Lower Dominium (mainly *Sargassum vulgare* and occasionally *Padina gymnospora*, *Dictyopteris delicatula* and *Grateloupia doryphora*). These dominiums cannot be recognized as “bands” or continuous “zones” in a landscape view of the community. Otherwise, these bands could be observed on a small scale on each large quarry stone.

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#### **TUBE SIZE COMPARISON AND AUTOMATED GROWTH MEASUREMENT IN A TUBULAR REACTOR USING *SPIRULINA PLANTENSIS***

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A tubular photobioreactor for outdoor cultivation of *Spirulina platensis* was successfully operated for the last two years. The reactor was made of transparent 2.4-cm diameter tubes with a total length of approximately 101-m and a volume of 124-liter. Flow was induced using an airlift pumping system. To optimize the system further, a larger tube diameter was also tested. Preliminary results have suggested that a larger tube diameter might provide increased output with reduced surface area requirements. Results of experiments comparing the productivity of the same culture volume in tubes of 2.4 cm and 5.0 cm will be presented which show a small decrease in productivity by volume, but a large increase in areal productivity. This suggests that the larger tube diameter would be an appropriate choice for larger scale systems. Additionally, data will be presented demonstrating the effectiveness of an on-line surface scatter turbidimeter for accurate measurement of *Spirulina* density when correlated to manual dry weight measurements.

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#### **THE FISH POND EXPERIMENT: CHAPTER TWO**

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Forty years ago, after 1 1/2 years of weekly surveys, a Baltimore fishpond was inoculated with three identifiable clones of *Pandorina morum*. The results of subsequent recollection attempts are published in *Amer. Naturalist* 118:761 (1981). More recently, molecular analyses have permitted characterization of the *P. morum* population that was endemic to the pond. The results, and their comparison with those from other *P. morum* sites, challenge some longstanding assumptions concerning the importance of various lifehistory stages to the introduction and maintenance of algal populations in temperate freshwater locales.

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#### **INFECTIOUS DISEASES IN MACRO-ALGAE: THE EFFECT ON HOST FITNESS**

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The understanding of infectious diseases of algae has improved significantly in recent years, particularly in the area of recognition and signaling, key processes that determine the success or failure of host invasion by the pathogen. Ecological studies have also contributed to better understanding the role of diseases in wild stands of the affected hosts. An aspect that has received only limited attention is the effect of the infections on host fitness, and in this context, we report a first attempt to quantifying the effects of *Pleurocapsa* sp. (Cyanophyta) on the reproductive potential of its host *Mazzaella laminarioides* (Rhodophyta). Infections by *Pleurocapsa* trigger the development of tumors that can result in major changes in frond morphology and texture. Two populations of the host were considered in the study. Our results indicate that infections do not cause a significant effect on the density or quality of the reproductive structures (i.e. cystocarps and tetrasporangia). However, the number of spores, settlement rates, germination success and offspring survival, were all affected negatively by the endophytic infections. The reported information and field-collected data, strengthen the notion that pathogens of algae may exert strong effects on their hosts at several levels, including reproduction. These effects can vary from host death during infections by highly pathogenic organisms to more subtle effects like those observed in the studied pathosystem. Infections by less aggressive pathogens, however, still may determine important effects at the population level by inducing differential mortality and reproductive success in infected individuals.

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### THE IMPACT OF DIFFERENTIAL GRAZING BY PHAGOTROPHIC CILIATES ON PHYTOPLANKTON BIOMASS AND COMMUNITY STRUCTURE

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There is a growing understanding that phagotrophic ciliates are often important members of aquatic communities in terms of their trophic role and mobilization of small cell production to higher consumers. As formidable consumers of small phytoplankton species they are likely to be also important in determining the community composition of the pico- and nanophytoplankton assemblages. Dilution method experiments were conducted during the winter and summer in the South Slough, an arm of the Coos Bay on the southern Oregon coast, to assess the impact of ciliate grazing on two size fractions of chlorophyll (0.2 to 5 mm and >5 mm) and on the growth and abundance of specific phytoplankton groups, particularly cryptophytes and *Synechococcus* sp. The premise of the dilution technique is that grazers are diluted with their food and the observed rate of change in chlorophyll or phytoplankton abundance is linearly related to the dilution factor. Results from previous studies using the dilution technique have been given in terms of the grazing impact of microzooplankton on total chlorophyll. The findings of the research presented using a more rigorous application of the dilution method suggest that ciliates are differential in their grazing of phytoplankton, targeting small phytoplankton biomass and preying selectively on components of the assemblage that constitute this biomass.

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### REACTIVE OXYGEN METABOLISM IN THE TROPICAL BROWN ALGA *DICTYOTA DICHOTOMA*

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We studied reactive oxygen metabolism in natural populations of the brown alga *Dictyota dichotoma* growing on coral reefs on the west coast of Barbados in March 2000. Algae from deep water (27 to 30 m) had lower dark respiration (0.08 cf. 0.21 mmole O<sub>2</sub> cm<sup>-2</sup> h<sup>-1</sup>), light compensation point (7 cf. 18 mmole photons m<sup>-2</sup> s<sup>-1</sup>) and gross light-saturated photosynthesis (0.44 cf. 0.68 mmole O<sub>2</sub> cm<sup>-2</sup> h<sup>-1</sup>) than the same spe-

cies from shallow (2 to 3 m) water. Compared to shallow water algae, those from 27 to 30 m were more susceptible to photoinhibition, produced more reactive oxygen (as measured by dichlorofluorescein production) and suffered lipid peroxidation when exposed to surface solar radiation. These data could not be explained by differences in activities of reactive oxygen processing enzymes. Although activities of ascorbate peroxidase (APX) were higher in shallow water algae, the opposite was true for superoxide dismutase (SOD) and catalase. *In situ* reactive oxygen production in shallow water *D. dichotoma* varied over the course of the day on both a sunny and overcast day, and was proportional to incident light and photosynthetic electron turnover rate (ETR) determined with a submersible modulated fluorometer. The natural population did not appear to experience lipid peroxidation. Activities of SOD and APX remained constant over the light period, whereas catalase declined in the afternoon on the sunny day, but not on the overcast day. Overall, our data demonstrate that algae produce reactive oxygen in nature under non-stressed conditions and moderate light (< 300 mmole photons m<sup>-2</sup> s<sup>-1</sup>) and that this occurs independently of the activity of catalase.

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### KELP ECOLOGY AND THE POINT LOMA KELP FOREST: A BLEND OF THE OLD AND THE NEW

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The history of the Point Loma kelp forest parallels that of the earlier pioneers in Europe, with an early descriptive focus moving quickly toward mechanistic studies. At Point Loma, research was initiated because of large-scale loss of kelps, so from the beginning there was an urgency to restore the kelp forest with a concomitant focus on sea urchin grazing. We review the evolving relationships between new questions and long-term observations that emphasize both small and large-scale events in time and space. For example, the early descriptive work grew into small-scale experimental work that demonstrated problems of scaling up from experiments. As we repeated experiments over larger spatial and temporal scales we learned that the low frequency variation at both scales is extremely important and must be understood before small-scale results are generalized. Regime shifts in the physical environment and the increasing effects of fishing represent continuing challenges to the kelp community structure that make it critically important to consider scaling problems.



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**GROWTH AND ULTRASTRUCTURAL CHANGES IN *DICTYOSPHAERIUM PULCHELLUM* EXPOSED TO COPPER (CHLOROCOC-CALES, CHLOROPHYCEAE)**

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In an assay addressing the toxicity of copper to *Dictyosphaerium pulchellum*, a 48 hours' exposure of axenic cultures of the alga to metal concentrations ranging from 0 to 300 mg L<sup>-1</sup> caused a significant decrease in the growth rate and cell chlorophyll content. Extracellular mucilage removal by controlled sonication before exposure to copper emphasized chlorophyll content reduction, but not cellular density diminution. Because mucilage acts as a cation interchanger, these results suggested that, during at least the first 48 hours, division rates might be affected by lower Cu<sup>+2</sup> concentrations than chlorophyll content. Ultrastructural observations showed that the normal organization of the chloroplasts was altered; they presented dilated lamellae, irregularly arranged with practically no stacking. Copper precipitates were mainly observed outside the plasmalemma or within the extracellular mucilage. Electron probe microanalysis confirmed these observations, showing only minor precipitates inside the cells.

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**TEMPORAL AND SPATIAL PRODUCTION OF AGGLUTININS IN MARINE MACROALGAE FROM THE MEXICAN CARIBBEAN**

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The presence or absence of secondary metabolites in algae has been long discussed. There are several hypotheses to explain the synthesis of secondary metabolites in algae as a response to herbivory pressure, competence or predation. In this work we made a screening test of chlorophyte and rhodophyte species collected in different sites of the Caribbean sea in order to test them for agglutinins. The extracts were prepared in a phosphate buffer solution 100-mM, pH 7.2 and filtered using 0.22 µm Millipore filters. Agglutination tests were done in microtiter-plates using for-

malinized rabbit and human A, O, B erythrocytes. Agglutination titer was recorded and expressed as the reciprocal of the highest dilution showing positive results. A total of 31 samples were analyzed; seven Chlorophyta species and 11 Rhodophyta showed agglutinating activity. The species that showed activity variation included the chlorophytes, *Caulerpa cupressoides*, *Caulerpa paspaloides*, *Halimeda opuntia* and *Penicillus capitatus* and the rhodophytes, *Chondria litoralis*, *Digenea simplex*, *Gracilaria cornea* and *Laurencia obtusa*. The agglutinating activity of *Liagora farinosa* is reported for the first time. This research indicates that ecological pressures are an important factor in seaweeds agglutinins synthesis.

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**GRAPPLING WITH CONFLICT AMONG INFORMATION SOURCES IN RECONSTRUCTION OF THE EARLY EVOLUTION OF LAND PLANTS**

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Although a close relationship between embryophytes (land plants) and charophycean green algae has been discussed for over a hundred years, the precise nature of this relationship remains uncertain. This is largely because of difficulty reconstructing the phylogeny of the basal members of this group. Recent analyses of SSU rDNA, *rbcL*, and concatenated chloroplast genes have all produced different phylogenies, and none is fully compatible with morphological data. Noteworthy conflict is apparent in the positions of the unicellular flagellate *Mesostigma* and the filamentous epiphyte *Chaetosphaeridium*. Several phenomena could result in such incongruence, including problems with the underlying data (taxon ID, sequence determination, alignment, etc.), choice of analytical method, lack of resolution with one or more of the datasets, unrecognized paralogy, and horizontal gene transfer. We have examined each of these possible sources of incongruence, and have determined that several factors underlie the apparent conflict among phylogenies. When these factors are taken into account a consensus molecular phylogeny begins to emerge. Despite the long divergence time in question, the prospects for reconstruction of land plant phylogeny are good.

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**EFFECT OF NITROGEN ADDITION ON A MIXED SPECIES PHYTOPLANKTON BLOOM**

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The Laguna Madre of Texas is a hypersaline lagoon that in recent years has been dominated by the brown tide alga, *Aureoumbra lagunensis* although a *Synechococcus*-like sub-dominant is typically present. Dominance of *A. lagunensis* is thought to be due at least in part to its nitrogen competitive abilities. A mesocosm study was performed to determine if an increase in ambient nitrogen concentration would lead to a shift in the dominant alga. Twelve fiberglass cylinders enclosing 1.2 cubic meters of Laguna Madre water were deployed for 16 days. Four times during this period, ammonium was added (N+ mesocosms) to half the mesocosms to achieve a post-addition concentration of approximately 40 mM. The average initial particulate N/P ratio was 40/1 (SD = 2.9) which recent evidence indicates is within the range of N/P ratios for N-limited *A. lagunensis*. In control mesocosms, total cell biovolume (TCB) of *A. lagunensis* cells dropped after 4 days by a factor of four and then increased to a level slightly above the day 0 value by day 16. In N+ mesocosms, *A. lagunensis* TCB was unchanged after 4 days then doubled by day 16. Despite the differences in final yield, growth rates of *A. lagunensis* in the two treatments were similar. The *Synechococcus*-like organism, showed a four-day lag before TCB in both treatments increased although at a slower rate in the control mesocosms. By the end of the experiment, TCB of *A. lagunensis* was two to six-times greater than that of sub-dominant. Despite the nitrogen treatment, *A. lagunensis* retained dominance.

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#### PHYTOPLANKTON COMMUNITY STRUCTURE: TEMPORAL VARIABILITY IN A TROPICAL UPWELLING ECOSYSTEM

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The Cariaco Basin (Southeastern Caribbean Sea) is the largest anoxic basin of oceanic character. Its sur-

face waters are affected by coastal upwelling during boreal winter and spring. Historical information on the phytoplankton communities in the basin is scarce. In November 1995, an oceanographic time-series station was established at 10.5°N - 64.66°W. In this study changes in the structure of the phytoplankton community in the upper 100-m layer were studied. Monthly samples to determine phytoplankton abundance and pigment composition (HPLC) were collected from November 1995 to January 1999; water temperature was used as proxy for upwelling. Surface waters reached temperatures  $\leq 24^\circ$  C during the upwelling season, and surpassed  $26^\circ$  C during the rest of the year. A total of 300 species were found. Generally, the highest number of cells ( $>500$  cells ml<sup>-1</sup>) were measured from January to April every year. Diatoms were the dominant group in terms of abundance, species composition (168 species) and pigments (Chl *c* and fucoxanthin) during this period. The abundance and marker pigment concentration (peridinin, but- and hex-fucoxanthin) for other groups support this observation. During the rest of the year diatom and total abundance decreased markedly ( $<100$  cells ml<sup>-1</sup>). Small organisms ( $<5$   $\mu$ m) became dominant. The maximum concentration values of zeaxanthin and But- and Hex-Fuco in this period indicated the presence of cyanobacteria and prymnesiophytes. The sparse and relatively low concentration of Chl *b*, lutein and prasinoxanthin indicated that chlorophytes and its allies are a minor floral component.

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#### ADVANCES IN A MONOGRAPH OF THE GENUS *GRACILARIA* (RHODOPHYTA) IN THE MEXICAN ATLANTIC

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Some species of the genus *Gracilaria* Greville (Gracilariales, Rhodophyta) are the main source of agar. For that reason, the taxonomic study of the taxon has become relevant for many scholars. In order to bring the Mexican floristics knowledge of the genus into the state-of-the-art about it, we have initiated the needed monography. The character definition has proved to be the most important task. We have found that the most reliable characters for the species delimitation are: general form of the thallus, length and width; form of the axes, length and width; branching pattern; number of axes from the holdfast; form of the holdfast; presence of constrictions at the base of the branches or not; color; length of the segments; consistency; form of the apices; length and thickness of the stipes; surface texture of the

branches, smooth, crispitous, etc; adherency to herbarium paper; cellular forms of the transition from cortex to medulla, size and thickness of cellular walls of the cells involved; number of cellular layers of the cortex, subcortex (if present) and medulla; form, number and distribution of the spermatangial conceptacles; form and size of the cystocarps, if constricted at the base or sessile; diameter of the ostiole and if it is apiculated or not; disposition of the carposporangial chains; form of the gonimoblastic cells, and form and number of tetrasporangia. A diagrammatic description for each is provided.

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#### PHYLOGENETIC SELECTION OF A RESOURCE: A NEW USE FOR CLADISTICS

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A phylogenetic model for the selection of commercial resources using the cladistic method is proposed. The group selected as an example was the marine agarophyte red algal genus *Gracilaria* Greville. We suggest the use of the cladistic principle of evolutionary transformational series in order to test the quality of agars instead of the assay-herror traditional method that consumes time and budget. If we assume that the "good quality of agar" in extant taxa is a sinapomorphic character (but not a reliable taxonomic one), then taxa included in the same monophyletic clade in which the species with "good quality of agar" are, has a high evolutionary possibility to share that character. In order to do this we have to incorporate to the set of available specific characters, those of the taxa actually used as a agar source but not present in the area under scope. A complete set of the basic cladistic data required for run the most popular program currently in use (PAUP) are provided. We applied the model to the Mexican Atlantic species and found that, using *Gracilaria chilensis* and *G. cornea* as "indicator taxa," and found Mexican populations of *G. crassissima*, *G. caudata*, *G. cervicornis* and *Gracilariopsis lemaneiformis* are candidates for a study of yield and agar properties.

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#### POTENTIAL TOOLS FOR TRACKING OCEAN CLIMATE: VARIABILITY IN STABLE ISOTOPES IN LIVING CORALLINE ALGAE

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Our ability to track long term climate change in coastal regions is limited in temperate and polar regions. Physical oceanographic dynamics in temperature and upwelling events can be recorded as carbon and oxygen stable isotope signals in carbonate producing organisms. Because coralline algae photosynthesize, produce calcium carbonate and are widely distributed, they may provide a new tool for detecting short-term change. However, little is known about how coralline algae incorporate stable isotopes into their calcite thallus structure. The objectives of this study were to determine if growth and isotopic signature differ in articulated coralline algae grown in different oceanographic regimes in Monterey Bay. The articulated alga *Calliarthron cheilospororioides* was outplanted at three locations varying in seawater temperature and upwelling strength. New algal growth was measured by staining the algae with Alizarin Red and enumerating the amount of accumulated material at the branch tips. Growth rates varied seasonally and spatially. Low-upwelling daily growth rates averaged 0.044–0.056 mm day<sup>-1</sup>, while high-upwelling growth rates were 0.083 mm day<sup>-1</sup>. Isotope ratios were obtained by analyzing microsampled portions of the alga in a mass spectrometer. Changes in the <sup>18</sup>O/<sup>16</sup>O and <sup>13</sup>C/<sup>12</sup>C ratios appear to reflect change in seawater temperature and upwelling strength, respectively.

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#### PRELIMINARY ANALYSIS OF QUANTITATIVE GENETICS AND PHENOTYPIC PLASTICITY IN *AULACOSEIRA SUBARCTICA* (BACILLAR-IOPHYTA)

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Several clones of *Aulacoseira subarctica* were isolated from Yellowstone, Lewis, and East Rosebud Lakes (Montana, Wyoming). Two to four clones from each lake were grown in batch cultures under three light intensities, 2, 11.4 and 115  $\mu\text{E m}^{-2} \text{s}^{-1}$ . Clones were conditioned to their light environment for a three-week period. Inoculants from the conditioned clones taken during log phase of growth, were grown until in log phase, then samples were collected. Five randomly chosen valves for 2 replicates of each clone were examined using a scanning electron microscope and captured on film at a magnification of 20,000x. Each image was digitized and quantitative morphometric characters were measured. A preliminary quantitative genetic analysis was performed on selected characters within each light environment. Plasticity of characters within clones across the three light regimes were also examined. The amount of variability found within characters in *A. subarctica* will be discussed in terms of environmental, genetic, and microenvironmental sources.

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### SCALE-DEPENDENT PATTERNS OF DISTURBANCE AND RECOVERY IN GIANT KELP FORESTS

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We studied spatial variability in giant kelp (*Macrocystis pyrifera*) forests at 84 sites along the west coast of North America in order to assess the impacts of the 1997–98 El Niño. Our sites spanned the geographic range of giant kelp in the Northern Hemisphere and were surveyed just before, immediately following, several months after, more than one year after, and nearly two years after the El Niño. Interspersion of sample units allowed us to compare the effects of this disturbance among spatial scales ranging from a few meters to more than a thousand kilometers. Variance components analyses revealed that El Niño shifted the relative importance of factors that regulate giant kelp communities from factors acting at the scale of a few meters (local control) to factors operating at hundreds of kilometers (regional control). Moreover, El Niño resulted in a near-to-complete loss of giant kelp populations throughout nearly two-thirds of the species' range. Evaluation of these effects along with oceanographic data (at the "appropriate" spatial scales), along with closer examination of giant kelp populations in the most severely impacted region (Baja) suggested that the among-region differences in giant kelp survival was due, at least in part, to El Niño-induced differences in ocean climate. Giant kelp recovery following El Niño was also scale-dependent, but driven by factors different from those of the disturbance. Here, we present results for several species of macroalgae in an attempt to relate the importance of El Niño to that of other processes in creating scale-dependent patterns of variability.

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### VARIATION IN TOXIN PRODUCTION OF SYNCHRONIZED CULTURES OF *CHRYSOCHROMULINA POLYLEPIS* (PRYMNESIOPHYTA)

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In European coastal waters toxic blooms of Prymnesiophytes occur regularly. *Chrysochromulina polylepis* is a highly toxic member of this taxonomic group, which formed a devastating bloom in Scandinavian waters in 1988. Blooms like these can cause severe damage to

benthic and pelagic communities and can result in million dollar losses for commercial fisheries and mariculture of fin and shellfish. Effective prevention and mitigation strategies require an understanding of environmental factors that contribute to the formation of harmful algal blooms, as well as information on the regulation of algal toxicity at the molecular level. To approach the latter aspect, we investigated the variation in toxin production of synchronized *C. polylepis* cultures by means of a sensitive bioassay and by flow cytometry. The results so far obtained show significant variation in cellular toxin content and specific toxin production throughout the sampling period of three days. The existence of times in the cell cycle of high and low levels of toxin production will be used as the basis for the identification of differentially expressed genes and proteins using differential display and 2-D protein gel electrophoresis. Preliminary results concerning the molecular analysis will be presented as well.

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### CO-OCCURRENCE OF CARRAGEENANS AND AGARIDS IN *GYMNOGONGRUS TORULOSUS* (GIGARTINALES, PHYLLOPHORACEAE) FROM ARGENTINA

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The red seaweeds, *Gigartina skottbetgii* and *Sarcothalia crispata*, have commercial value as raw material for the industrial production of phycocolloids (carrageenans) in Argentina. The third alga with potential possibilities for the carrageenan production is *Gymnogongrus torulosus*. Herein, we report the study of the polysaccharide system present in *Gymnogongrus torulosus*, which contribute to the estimation of the importance of this algae in the seaweed industry. Analysis of the hot water-soluble extract (C1), by FT-IR, methylation and <sup>13</sup>C NMR, showed mainly the presence of iota/kappa carrageenan hybrid (molar ratio ~ 2:1). The soluble fractions obtained after KCl fractionation (F3, 16.1 % of C1) and soluble after alkaline treatment and KCl fractionation (F3T3, 34.6% of F3T) gave negative optical rotations (-15.5 and -55.4, respectively), considerably lower than those reported for kappa/iota carrageenans (from 56.1 to 66.5). These fractions (F3 and F3T3) have significant quantities of L-galactose (11.1% and 29.8%) and L-3,6-anhydrogalactose (19.2% and 4.3%). The direct relationship between the optical rotation and the percentage of L-galactose indicated that its structural influence is similar in all the fractions. The results suggest that *Gymnogongrus torulosus* biosynthesizes a polysaccharide system with co-occurrence of carrageenans and agaroids in the same thallus.

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### PHOTOSYNTHESIS IN THE DEEP BLUE SEA: PHOTOPHYSIOLOGICAL RESPONSES OF *PHAEODACTYLUM TRICORNUTUM* TO DYNAMIC AND STATIC IRRADIANCES

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The kinetics of xanthophyll-cycle pigment switching and fluorescence quenching dynamics in the marine diatom, *Phaeodactylum tricornutum* were determined in the context of dynamic and static growth light. Cultures were grown in a modified photobioreactor capable of producing dynamic light fields which exhibited attenuation characteristics similar to that of water; these cultures were pre-acclimated to high and low, static and dynamic, growth-light regimes for at least three days, and then examined under high, static and dynamic light. Pigment pools varied markedly. The two static light cultures had pigment complements that were very similar to "traditional" high and low-light static cultures. The dynamic-light grown cultures had pigment complements, which were very similar to each other but different from the static-grown cultures. The maximum xanthophyll-cycle pigment de-epoxidation state attainable under saturating light was equal for all four treatments. Induction of fluorescence quenching was significantly faster in the static-grown cultures, while xanthophyll-cycle de-epoxidation rates did not show as much variation. Minimum irradiances for xanthophyll-cycle induction were correlated to average growth irradiance. Taken as a whole, the results from this work suggest that dynamic light-grown phytoplankton have a unique photosynthetic functionality that is different from static light-grown phytoplankton. The significance of these observations in the context of realistic light fields, and the photosynthetic response capabilities of algae grown under them will be discussed.

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### HETEROGENEITY OF CHLOROPLAST GAPDH GENES IN THE DINOPHYCEAE

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Dinoflagellates provide valuable models for studying endosymbiosis and chloroplast evolution. While many species are heterotrophic, photosynthetic species containing plastids of different evolutionary origin have

been isolated, indicating that dinoflagellates acquired chloroplasts multiple times during evolution. Furthermore, dinoflagellates contain nuclear genes encoding chloroplast proteins that are not typically found in eukaryotes. These include the type II form of RuBisCO, and a eubacterial form of glyceraldehyde-3-phosphate dehydrogenase (GAPCp) that we have recently characterized from *Gonyaulax polyedra*. The evolutionary origin of this GAPCp isoform is unclear. It is phylogenetically distant from the *Gonyaulax* cytosolic form (GAPC) and from the chloroplast forms (GAPA/B) of cyanobacterial origin typically found in photosynthetic eukaryotes. It has previously been described only from cryptomonads, raising the possibility that *Gonyaulax* acquired the gene through lateral transfer. We have addressed this issue by examining the phylogeny of GAPDH genes from other dinoflagellates. We have isolated genes from *Gymnodinium mikimotoi* and *Pyrocystis lunula*. The predicted protein sequence of the *G. mikimotoi* gene is 47% similar to *Gonyaulax* GAPC and GAPCp and phylogenetic analysis places them in the same eubacterial clade. The predicted amino acid sequence of the *P. lunula* gene is 41% and 37% similar to *Gonyaulax* GAPCp and GAPC isoforms, respectively. However, phylogenetic analysis groups it within the clade comprising cyanobacterial and eukaryotic GAPA/B isoforms and shows that it is closely related to the chloroplast form of *Euglena gracilis*. Thus, within the Dinophyceae, GAPDH genes of different evolutionary origin have been recruited to participate in chloroplast metabolism.

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### CLONING, SEQUENCING AND EXPRESSION OF A HISTONE-LIKE PROTEIN FROM THE PHOTOSYNTHETIC DINOFLAGELLATE *GONYAULAX POLYEDRA*

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The presence of small basic DNA-binding proteins, or histone-like proteins (HLPs), in dinoflagellate nuclei has been well documented. Genes for HLPs have been cloned from *Cryptocodinium cohnii* (HCc 1 and 2) and *Alexandrium fundyense* (Haf) but their predicted protein sequences show no homology to histones from other eukaryotes or to bacterial HLPs. The precise role of these HLPs in dinoflagellate nuclei is uncertain; the HLP:DNA ratio is too low to facilitate packaging of the entire (very large) genome. In order to investigate the role of these proteins we have cloned a gene for a HLP from *Gonyaulax polyedra* (HGp). Unlike the genes from *C. cohnii*, the *G. polyedra* gene contains no introns. It codes for a protein with a predicted mo-

lecular mass of 10,985 Da, and an isoelectric point of 10.5. We over-expressed this protein in *Escherichia coli* using a construct that expresses the full-length protein with an N-terminal histidine tag, and purified it by Nickel-affinity and cation-exchange chromatography. While the predicted molecular mass of the fusion protein is 12,973 Da, similar to sizes observed for acid-soluble *G. polyedra* nuclear proteins, the relative mobility on denaturing SDS-polyacrylamide gel electrophoresis is 20,000 Da. We attribute the slower mobility in denaturing page to the basicity of the protein and predict that the relatively faster mobility exhibited by native HGp is due in part to post-translational modification of basic residues. Such modifications have been observed in histones and play an important role in the regulation of transcription in eukaryotes, a function that HLPs may assume in dinoflagellates. This possibility is being investigated.

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#### **BIODIVERSITY OF PLANKTONIC DINOFLAGELLATE SPECIES IN MANGROVE PONDS, PELICAN CAYS, BELIZE**

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Information on the population structure of planktonic dinoflagellates is reported in the coral reef-mangrove ecosystem at Pelican Cays, Belize. Six sites examined included: Cat Cay, Douglas Cay, Elbow Cay, Fisherman's Cay, Lagoon Cay and Manatee Cay. A spectacular and rich dinoflagellate taxa including oceanic, coastal and offshore species are illustrated. The presence of oceanic species in the studied cays is an unexpected observation since dinoflagellate assemblages are virtually enclosed within ponds bordered by coral ridges that limits water exchange with the open ocean except during storm events. I am also reporting significant differences in the dinoflagellate associations among the studied cays. Dominant taxa included 16 *Proroperidinium* species, 11 *Gonyaulax* species, and ten *Ceratium* species. Only six planktonic species were harmful. Bloom forming species included *Ceratium furca* and *Gonyaulax polygramma*. A much more diverse autotrophic and heterotrophic dinoflagellate population characterizes the Pelican Cays than previously suspected. Some species are reported the first time: *Proroperidinium belizeanum* sp. nov., *P. pyriforme* Balech, *P. steidingeriae* Balech, *P. depressum* (Bailey) Balech, and *P. divergens* (Ehrenberg) Balech. These results demonstrate that the Belizean coral reef-mangrove ecosystem is a delicate and species-rich environment, and as such, should be protected and preserved.

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#### **ALGAE AS WATER QUALITY INDICATORS FOR A DYSTROPHIC POND**

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The physico-chemical, bacteriological, and algal composition of R.U.Bi.S. Pond were studied since March 1999. The pond, located in Cape May County, NJ, is a dystrophic body of water, with high sediment:water ratio and organic matter content creating anoxic conditions during fall and early winter. Water quality indicators based on periphytic algae and water quality indices that are specific to R.U.Bi.S. Pond are being developed. Such indicators could be used for similar bodies of water and for the restorability of this dystrophic ecosystem integrity. Two sampling sites were studied. Samples were collected weekly, using a periphytometer, for seasonal analysis of succession. Samples for water chemistry, bacteriology, and periphytic algae were collected bi-monthly. The predominant algal species found during summer '99 were *Phormidium*, *Oscillatoria*, *Chlorella*, and *Eunotia*. During fall '99, the predominant species were *Oscillatoria* and *Phormidium*. In winter, the community was dominated by *Chlorella* and *Chlamydomonas*. Species present year-round were *Nitzschia*, *Oedogonium*, *Eunotia*, and *Navicula*. Data analysis indicated variations among the two sampling sites, with total cell counts and species diversity higher at the northern site. Algal cell counts, chlorophyll *a* concentrations, species diversity, and D.O. levels were greatest during summer. Biomass, hardness, HPCs and total organic carbon peaked during the fall. Nutrients, pH, and coliforms remained constant during the study period. The predominant algae found were pollution-tolerant genera, categorizing the pond as highly dystrophic. Species diversity indices indicated that water quality decreased during the fall/winter months, with the southern site being more environmentally stressed than the northern site.

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#### **PHYLOGENY, BIOGEOGRAPHY AND LIFE HISTORY EVOLUTION IN THE RED ALGAL FAMILY PHYLLOPHORACEAE (GIGARTINALES)**

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Red algae are exceptional for the great diversity in reproductive morphology and for their complex life histories. In particular, the family Phyllophoraceae, consisting of ~100 species worldwide, stands out in exhibiting a wide spectrum of unique life history types that makes it unusually interesting for assessing the

phylogenetic importance of reproductive traits relative to classification criteria. Type of life history and position of the reproductive structures on the plant body have traditionally formed the basis for separating eleven genera in the Phyllophoraceae; however, phylogenetic analyses inferred from three sets of DNA sequences [chloroplast-encoded *rbcL*, nuclear large-subunit ribosomal RNA gene (LSU), and internal transcribed spacer regions (ITS) of nuclear ribosomal DNA], instead indicate a lack of correlation between type of life history and phylogenetic relationships among the established taxa. This lack of correlation dramatically challenges all of the traditional taxonomy. The study will answer the question which morphological features and which aspects of life history evolution can be used as meaningful indicators of phylogenetic relationships in the Phyllophoraceae. The results are addressed in light of global biogeographic hypotheses for the family.

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#### **CHARACTERIZATION OF THE OFFSHORE MARINE MACROALGAE FROM THE NORTH-WESTERN GULF OF MEXICO HARD BANK COMMUNITIES**

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The upper northwestern Gulf basin is characterized by a relatively shallow but well-developed continental shelf with an extensive system of mid- and outer shelf hard banks of varying origin and composition. Observations done by our laboratory while participating in monitoring cruises to the National Marine Sanctuaries of the East and West Banks of the Texas Flower Gardens, Stetson Banks (TX) and Sonnier Banks (LA) reveal a surprisingly different floristic composition among the sites. Several range extensions, new macro-algal records for the Gulf of Mexico, and new species imply a much more complex Algal Zone community than the one proposed by Rezak *et al.* (1985). Our ongoing project includes the development of a modern taxonomic, phylogenetic, morphological and genetic database essential to informed management for preservation of biological diversity. It also provides a framework of seasonal algal composition against which long-term trends and anomalies in algal distribution and health of the Gulf of Mexico can be assessed at future dates.

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#### **LIGHT HARVESTING ANTENNAE SYSTEMS: LESSONS FROM SIMPLE ALGAE, THE ROLE OF SCIENTISTS, AND TRENDS**

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Algae, with their variety of pigmentation and extant evidence of primary to tertiary endosymbiotic states, present rich opportunities for exploring evolutionary relationships. A strong link is suggested by the conservation of phycobilisome structure between red algal chloroplasts and cyanobacterial progenitors. Although phycobilisomes are the major antennae of photosystem II reaction centers in both groups, a chlorophyll (Chl)-carotenoid light-harvesting complex (LHC) evolved in rhodophytes around the photosystem I reaction centers. LHC-polypeptides of all eukaryotic algae and green plants have high conservation of 8 putative Chl-binding sites on three trans-membrane helices, yet many different types of Chls and carotenoids serve in light harvesting in diverse algal groups. In view of the phylogenetic relatedness of LHC polypeptides (Durnford *et al.* 1999, *J. Mol. Evol.* 48:59) the Chl and carotenoid-binding capability was examined with pigments from diverse algae. A recombinant *P. cruentum* polypeptide, LHCaR1, reconstituted with its own pigments functionally bound about 8.0 Chl *a*, and 4.0 zeaxanthin, comparable to native its polypeptides. Upon reconstitution with diatom pigments 7 Chl *a*, 1 Chl *c*, and 10 xanthophylls were bound per polypeptide, which participated in energy transfer to Chl *a*. Spinach pigments also bound to the polypeptide, incorporating a total of 8 Chl (both *a* and *b*) and ca. 4 xanthophylls, with energy transfer from lutein and Chl *b* to Chl *a*. These results suggest that the red algal LHCs retained the flexibility to bind different types of Chls and carotenoids. Such an ancestral feature may have contributed to the successful radiation of photosynthetic algae into varied light environments.

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#### **CURRENT STATUS OF THE INVASIVE GREEN ALGA *CODIUM FRAGILE* IN EASTERN CANADA**

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Ten years after the initial discovery of *Codium fragile* in eastern Canada on the Atlantic coast of Nova Scotia, *C. fragile* has extended its range considerably to northern Nova Scotia, southern New Brunswick and Prince Edward Island (P.E.I.) in the Gulf of St. Lawrence. In all principal areas *C. fragile* subsp. *tomentosoides* is present. In two regions of Prince Edward Island, *Codium* is becoming extremely abundant, and is known by local fishers as the "oyster thief". A potential second taxon of *Codium* was collected in 1999 at three sites in Malpeque Bay where it is locally abundant. These plants are distinguished from *C. fragile* subsp.

*tomentosoides* by their smaller frond size, flatter utricle tops, smaller mucrons, and smaller gametangia. Principal component analysis distinguishes plants of the two types. The abundance of *C. fragile* on both hard bottom and soft bottom substrata suggests that it will become the dominant alga in many subtidal algal communities in eastern Canada.

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### BIOGEOGRAPHY AND ECOLOGY OF THE KELP/RED ALGAL SYMBIOSIS

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A kelp/red algal symbiosis is described from nature based on extensive collections from the San Juan Islands, Washington. Kelp gametophytes were found as endophytes in the cell walls of seventeen species of red algae in three different kelp communities. Host red algae were mostly filamentous (e.g., *Pleonosporium vancouverianum*) or polysiphonous (e.g. *Polysiphonia paniculata*). The kelp gametophytes completed vegetative and reproductive development in the hosts with gametangia formed at the host surface and with sporophytes up to several mm in height being produced while still attached to the host. To date, none of the kelp gametophytes from nature have been identified to genus or species, although the gametophyte of *Nereocystis luetkeana* is a potential candidate for the symbiosis. Preliminary observations from Nova Scotia and the Isle of Man have not found the association in the Atlantic Ocean. Laboratory studies in Korea successfully reconstructed the symbiosis in the red alga *Aglaothamnion oosumiense* using zoospores of *Undaria pinnatifida* but not *Laminaria religiosa*. Here we outline the development of the symbiosis and discuss the potential adaptive significance of the kelp/red algal interaction.

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### PHYLOGENETIC AND BIOGEOGRAPHIC AFFINITIES OF THE HALYMENIALES AND RHODYMENIALES (RHODOPHYTA) FROM THE GULF OF MEXICO

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Generic and species concepts within the red algal assemblages Halymeniales and Rhodymeniales are discussed for taxa inhabiting the western Gulf of Mexico. Two principal biogeographic assemblages occur: an off-shore deep-water group (including *Coelarthrum cliftonii*, *Halymenia* spp., *Botryocladia* spp., *Gloiocladia*) representing remnants of a Tethyan distribution, and a near-shore intertidal flora (comprising *Rhodymenia*, *Grateloupia* and *Prionitis*) which instead reveals biogeographic affinities with the Carolinian Province. These two distinct marine floras will be contrasted phylogenetically based on DNA sequence analysis inferred from chloroplast-encoded *rbcL*. The generic descriptions of *Grateloupia* and *Prionitis* are in need of taxonomic revision based on a survey of species from Caribbean and Pacific Mexico.

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### FUNCTIONAL BINDING OF DIATOM PIGMENTS TO A RHODOPHYTE LIGHT HARVESTING POLYPEPTIDE

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A close relationship of light harvesting polypeptides (LHC) of rhodophytes, chromophytes and chlorophytes is inferred from the amino acid sequence similarity in three transmembrane helices, and from the conservation of 8 putative chlorophyll (Chl)-binding sites (Durnford *et al.* 1999, *J. Mol. Evol.* 48:59). Differences in Chl and carotenoid pigments have been a major classification feature. Thus, it was of interest to ascertain whether pigments from a diatom (*Thalassiosira fluviatilis*) could be functionally inserted into a red algal (*Porphyridium cruentum*) polypeptide. A recombinant polypeptide, LHCaR1, was reconstituted with pigment extracts from the diatom (Chls *a* and *c*, fucoxanthin, diadinoxanthin and  $\beta$ -carotene). The pigments were found attached to protein upon separation on sucrose gradients, and on non-denaturing gels. Absorption and fluorescence excitation spectra revealed individual peaks corresponding to the absorption maxima of Chl *a* at 438/672 nm; Chl *c* at 463/638 nm; and fucoxanthin at 493/540 nm. Fluorescence emission and CD spectra showed functional binding and suitable orientation for energy transfer from Chl *c* and carotenoids to Chl *a*. The LHCaR1 successfully folded in the presence of the heterologous pigments and bound 7 Chl *a*, 1 Chl *c*, 8 fucoxanthin, and 1.9 diadinoxanthin per polypeptide. By comparison, this polypeptide with *P. cruentum* pigments binds 8 Chl *a*, and 4 zeaxanthins, thus revealing its capability of functionally binding 8 Chls with variations in carotenoid numbers. Such a trait may have favored the diversification of a large



family of LHCs and the successful radiation of photosynthetic eukaryotes into different light environments.

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### THE SECRET LIFE OF KELPS: PLANKTONIC PROCESSES AND POPULATION DYNAMICS

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Understanding spatio-temporal variability in recruitment is vital to studies of kelp population dynamics. Research on settlement and post-settlement processes has suggested that arrival of kelp zoospores to suitable substrate is important in limiting kelp recruitment, yet the role of planktonic processes in kelp population dynamics has not been studied due to difficulties in sampling and identifying zoospores. I developed a method to estimate kelp zoospore abundance from *in situ* plankton samples and used it to study various processes regulating the availability of giant kelp (*Macrocystis pyrifera*) zoospores for settlement. My studies focused on (1) identifying temporal scales over which zoospore abundance is most variable, (2) describing physical and biological processes that regulate this variability, and (3) determining the relationship between zoospore abundance and settlement. I found that short-term variability in zoospore abundance (<24 hrs) was not due to changes in supply but rather dispersion, caused by oscillating hydrodynamic forces (e.g. waves). Long-term variability in zoospore abundance, however, was best explained by the size and density of reproductive adult plants, with zoospore abundance being most variable at the scale of days to months. Changes in adult reproductive condition caused rapid changes in zoospore abundance suggesting that the supply of kelp zoospores is sensitive to environmental regulation of adult physiology. Thus, unlike with marine animals, these results indicate that variability in kelp propagule supply, over scales most likely to affect subsequent settlement and recruitment, is more tightly coupled to demographic and reproductive mechanisms than to physical transport processes.

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### LET THERE BE LIGHT (BUT NOT TOO MUCH): MOLECULAR EVOLUTION OF LIGHT-HARVESTING COMPLEXES

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The light-harvesting antennas common to all photosynthetic eukaryotes belong to two protein families: the Chl a-binding core complex family (e.g. CP47, CP43), and the LHC superfamily, whose members

bind a variety of Chls. The former are all chloroplast-encoded and have high sequence similarity to their cyanobacterial homologs; the latter are all nucleus-encoded and have a much more complex relationship with each other and their (presumed) cyanobacterial ancestors. Phylogenetic analysis of the LHC superfamily protein sequences show that they follow the endosymbiotic history of their hosts and suggest that the ancestral cyanobacterial genes were copied to the nucleus soon after the establishment of the primary endosymbiosis. Both families have members whose role appears to be related to photoprotection rather than light-harvesting, suggesting that these families may have originated as stress-response proteins. In support of this, all members of the LHC superfamily bind significant amounts of carotenoid, and much of the variation between them resides in the bewildering array of different carotenoids they bind. In fact, it has been suggested that this family originated as carotenoid-binding proteins and only later acquired Chl (Gantt, E. 1998; *Photosynthesis: Mechanisms & Effects I*, pp. 239-246).

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### HIGH BREVETOXIN CONCENTRATIONS IN GYMNODINIUM BREVE BLOOMS ALONG THE NORTHWEST FLORIDA COAST DURING 1999

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Blooms of the dinoflagellate *Gymnodinium breve* (i.e. red tides) produce brevetoxins (PbTx) that negatively impact the Gulf of Mexico ecosystem, human health, and local economies. Characterizing and predicting bloom events and their impacts requires knowledge of *G. breve* abundance and PbTx concentrations in the water column. We report results from a bloom that occurred during the fall and winter of 1999 in NW Florida coastal waters. Data were collected from 16 stations on 3 sampling dates (29 Sept., 9 Nov., 1 Dec.), including basic hydrography, nutrient concentrations, *G. breve* abundances, and brevetoxin concentrations. *G. breve* cells were enumerated using flow cytometry and PbTx's were isolated from seawater using dichloromethane (DCM) partitioning. Brevetoxins were quantified by HPLC-DAD using a C-18 column and an acetonitrile-water gradient elution. Literature estimates of total PbTx concentration (PbTx's 1, 2, 3) of cultured and field-collected *G. breve* suggest a range in concentration from 7 to 17 pg cell<sup>-1</sup>. We measured total PbTx levels that greatly exceeded these values [Sept., 47-67 pg cell<sup>-1</sup> (n=5); Nov., 59-126 pg cell<sup>-1</sup> (n=3), Dec., 12-63 pg cell<sup>-1</sup> (n=8)]. PbTx-2 was the

predominant (67–75%) PbTx isomer found in these blooms. PbTx-1 and PbTx-3 were found at 11–22% and ND–28% of total PbTx, respectively.

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### EXTRACELLULAR MATRIX PROTEO-GLYCANS INVOLVED IN DIATOM ADHESION AND MOTILITY

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Although diatom extracellular matrices are usually thought of exclusively in terms of the beautiful, architecturally complex silicious frustule, polymers exuded through the frustule are critical mediators of interactions with the external environment. In several species, complex proteoglycans appear to be the primary components involved in adhesion and motility. When viewed with high-resolution cryo-scanning electron microscopy methods, the ubiquity and pervasiveness of these polymers was revealed in both freshwater and marine taxa. Monoclonal antibody mapping of carbohydrate epitopes characterized by NMR, methylation and monosaccharide analysis and correlated with structural observations by EM revealed an organizational pattern far more complex than previously proposed. Modeling assembly of extracellular “stalks” in the marine biofouling diatom *Achnanthes longipes* involves intracellular sequestering of multiple components, deposition at the protoplasmic membrane/diatotepum interface, transport through the multilayered diatotepum and holes in the silica, extrusion from the frustule, and assembly into a very complex multi-laminate biocomposite structure. The mechanism of extracellular polymer participation in motility is complex in a different way, as some current models of raphe associated motility involve cytoskeletal interactions and molecular motors.

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### CULTURES OF CHYTRIDS - PARASITES OF ALGAE IN BIOLOGICAL INSTITUTE OF PETERSBURG UNIVERSITY

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During our survey of chytrids occurring on algae in fresh waters in the northwestern part of the Russian Federation we never observed mass development of parasites, but if cultures of *Chlorococcum minutum* Starr or *Tribonema gayanum* Pasch. were inoculated with a sample of water (0.5 L), the parasites appeared in 10% of the cases. Two-clonal (alga and parasite) cultures were obtained by plating of the material on the lawn of algal cells on the solid medium with benzyl

penicillin. 22 cultures are now available. For the most part, cultures belong to *Rhizophyidium* species. *Chytridium lagenula* (Braun) sensu Scherffel is grown on *Tribonema gayanum* cells. Cultures differ in possible host range, where some have a very wide host range, but others grow only on the cells of *Chlorococcum minutum* (from 40 strains of different chlorococcalean algae examined). Only two *Rhizophyidium* spp. cultures are opportunists and grow on dead algal cells and organic media. Collection is used for the examination of interaction between algae and fungi.

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### IMPACT OF STRESS CONDITIONS ON THE PHOTOSYNTHETIC MACHINERY

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Organisms sense their environment and attempt to tune their metabolism to ambient conditions to efficiently utilize available resources. Cyanobacteria under conditions of stress (nutrient limitation, excess light) modify the photosynthetic apparatus by eliminating most photosystem II activity and degrading their light-harvesting complex or phycobilisomes (PBS). We have identified cyanobacterial mutants that cannot acclimate normally to nutrient deprivation. One of these mutants has a lesion in a gene encoding a small polypeptide designated NblA that appears to target the PBS for degradation during nutrient limitation. A second lesion is in the nblB gene, which encodes a polypeptide with homology to a family of lyases involved in attaching chromophores to apophycobiliproteins. This protein may remove bilin chromophores from phycobiliprotein subunits prior to their degradation. A third mutant is defective in nblR, which encodes a transcription factor that controls some of the “general” responses that occur during any of a number of different stress conditions. It is required for degradation of PBS and appears to be necessary for controlling photosynthetic activity during both nutrient limitation and high light conditions; this control is critical for survival of cells during conditions of environmental stress. A fourth mutant is altered in nblS, which encodes a sensor kinase that plays a role in regulating both nutrient stress and high light responses. This sensor protein has a PAS domain, appears to bind a flavin, and integrates the response of the cell to a variety of stress conditions; it may sense the redox status of the cell. I will present a model that describes how cyanobacteria sense and respond to environmental change.

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### MOLECULAR STUDY OF THE TOXIC ALGAE *DINOPHYSIS* SPP. FROM THE FRENCH COAST

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Dinoflagellates of the genus *Dinophysis* are agents of Diarrhetic Shellfish Poisoning (DSP). They occur along the French coast and affect shellfish exploitation during most of the year (during spring, summer and autumn). Because this species is difficult to cultivate, very little is known about this organism. The first problem is the species-delineation within this genus which is sometimes unclear based upon the solely on morphological features, in particular for the complex *D. acuminata* (*D. cf. acuminata*, *D. cf. norvegica*, *D. cf. sacculus*, and *D. skagii*) or the complex *D. sacculus* (*D. sacculus* and *D. pavillardii*). The second problem is its detection in natural samples. French *Dinophysis* blooms have been reported to be toxic under 100 cells L<sup>-1</sup>, a concentration which corresponds to less than 1 cell 10-mL<sup>-1</sup>, as determined by the Utermöhl method of enumeration. Molecular tools may help to resolve these two kind of problems. During one year (spring 1999 to spring 2000), more than 100 fixed samples containing *Dinophysis* spp. cells were collected along the French coast by the French monitoring network (or REPHY; <http://www.ifremer.fr>). The genetic diversity of *Dinophysis* spp. was studied by sequencing and analysis of ribosomal DNA genes. We found that sequences were highly conserved between species or within the *D. acuminata* or *D. sacculus* complex. Two oligonucleotide probes, specific to these complex groups, were designed. Their specificity and sensitivity are actually tested on natural samples by a PCR-based assay. Further investigation will include the development of standard molecular diagnostics due to their rapid and sensitive detection in natural samples.

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### GRACILARIA FROM THE GULF OF MEXICO, WITH SPECIAL EMPHASIS ON *GRACILARIA TIKVAHIAE* BASED ON TWO MOLECULAR DATASETS

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Despite a recent body of literature, the species of *Gracilaria* recognized for the Gulf of Mexico are still incompletely characterized, misidentified, and species limits not well defined due to huge morphological plasticity and overlapping habits. To better characterize these taxa and evaluate their taxonomic relationships, we provide a chloroplast-encoded *rbcl* phylogeny encompassing primarily Gulf, western Atlantic, and Caribbean species. Our results indicate the need to resurrect older, validly published names currently placed in synonymy under other species of *Gracilaria*, and to describe new species. For *Gracilaria tikvahia*, in particular, a combination of DNA sequences [*rbcl*, and internal transcribed spacer regions (ITS) of nuclear ribosomal DNA] from specimens collected across a wide biogeographic area (Nova Scotia, Canada, to Las Coloradas, Yucatan Peninsula) successfully promotes the integration and correlation of molecular phylogenies and morphology with biogeographic hypotheses.

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### PHYLOGEOGRAPHY AND WITHIN-STREAM GENETIC VARIATION OF *BATRACHOSPERMUM HELMENTOSUM* (RHODOPHYTA) USING INTER-SIMPLE SEQUENCE REPEATS

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A relatively new molecular marker technique, inter-simple sequence repeats (ISSR), was used to assess the within-stream reach genetic variation and phylogeography of the freshwater red alga, *Batrachospermum helmentosum*. The within-stream reach study was undertaken in Southeastern Ohio, where 58 individuals were collected from an approximately 200 m stream length. Individuals appeared to be congregated into three distinct areas, with no plants between areas. Using three primers, 100 polymorphic bands were scored. Although it was hypothesized that genetic variation would reflect physical distance, the results showed six distinct genetic groupings, some of which consisted of physically distant individuals. Overall, genetic diversity was greater among individuals than between geographic and genetic groups. To investigate phylogeography, 11 stream reaches (3 from Ohio and one each from Connecticut, Massachusetts, Rhode Island, North Carolina, Tennessee, Indiana, Michigan and Louisiana) were sampled throughout the alga's known distribution in North America. Approximately 15 individuals were collected per stream for ISSR analysis. Five primers, yielding 243 polymorphic bands, were used to examine variation among all individuals. Neighbor-joining trees generated from the Dice, Jaccard and Excoffier coefficients showed that individuals from the same stream formed a clade. Among streams there was no distinct correlation be-

tween geographic distance and genetic similarity. Genetic groupings varied slightly among distance coefficients employed. Michigan and Tennessee, Connecticut and Rhode Island, and Indiana and Massachusetts streams consistently showed the same relationship, but the relationships among the three Ohio streams and Louisiana stream were variably related to each other.

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### MACROALGAL TISSUE NUTRIENTS AS INDICATORS OF NITROGEN AND PHOSPHORUS STATUS IN THE FLORIDA KEYS

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This study used the tremendous biochemical and ecological diversity of macroalgae to assess nitrogen and phosphorus availability at a broad, ecosystem-level scale in the Florida Keys and nearby waters. Spatial variation in tissue nutrients (carbon, C; nitrogen, N; phosphorus, P) of dominant macroalgae were assessed, both as ratios and absolute values, along 12 inshore-offshore transects in the Florida Keys and at 10 stations in nearby Florida Bay. The resulting detailed analysis demonstrated spatial and temporal patterns in macroalgal tissue nutrients. The transect data revealed no universal inshore-offshore patterns in tissue nutrients and no obvious "hotspots" of nutrient enrichment. Similarly, when data were compared among segments, there was no universal geographical pattern in tissue nutrients for all species. The most striking result was that the N and P status of macroalgae in Florida Bay was significantly different than other locations. Macroalgae collected from Florida Bay generally had higher N and lower P levels than algae collected elsewhere. The most common inshore-offshore pattern was higher %N and lower %P availability inshore; however, limited inshore-offshore differences in N:P ratio suggests that both nutrients were generally readily available in proportional amounts required by the various species. Most species in this study had higher %N, and to a lesser extent, higher %P and %C in March than in July. Based on the published literature on other species of macroalgae, it appears that N and P are generally available in sufficient quantities that most macroalgal growth is not limited by either nutrient.

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### GRATELOUPIA DORYPHORA HAS ESTABLISHED RESIDENCY IN RHODE ISLAND WATERS

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Since 1994, when *Grateloupia doryphora* (Halymeniaceae, Rhodophyta) was first detected in Rhode Island, the species has spread to the northern portions of Narragansett Bay and onto the open coast of Rhode Island Sound. Specimens collected at 5 m depths off North Prudence Island reached 175 cm in length and establish this alga as the largest member of the Florideophyceae on North Atlantic shores. Percent cover of populations is seasonal: highest in fall (September through November) and lowest in spring (March through May). Monthly measurements at three stations in Narragansett Bay show significant seasonal differences ( $p < 0.01$ ) over two annual cycles. Artificial substrata placed in the field at known periods allowed measurements of growth rates on individual thalli. Laboratory culture clarified the sequence of life history stages that make this species a successful contender for space.

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### A REEXAMINATION OF THE SYSTEMATICS OF THE ACROCHAETIALES (RHODOPHYTA) USING LARGE-SUBUNIT RDNA SEQUENCE DATA

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The taxonomic history of the red algal order Acrochaetiales is chaotic. There is no consensus in the literature as to how many genera should be recognized or in the assignment of the over 400 species to these genera. Morphological and anatomical studies have provided a suite of possible characters to delineate genera within this order, but there have been major discrepancies in the assessment and use of these features. The phylogenetic placement of the Acrochaetiales has also been the focus of debate. Once thought to be the most ancestral florideophyte lineage, recent molecular systematic studies have illustrated that this order is a derived lineage closely related to the Nemaliales and Palmariales. Phylogenies using sequences of the small-subunit (SSU) rDNA have strongly supported two very divergent lineages within a possibly polyphyletic Acrochaetiales. The relationships between these two groups and among other closely related rhodophyte orders were not resolved. We have generated large-subunit (LSU) rDNA sequence data for representatives of the Acrochaetiales and related taxa. Distance and parsimony phylogenies based on LSU and combined SSU and LSU data will be presented. The increased phylogenetic signal afforded by this approach will shed light on previous conundrums in the systematics of this group.

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**INTRAGENOMIC nrDNA ITS POLYMORPHISM IN THE *STEPHANODISCUS NIAGARAE* COMPLEX (BACILLARIOPHYCEAE)**

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Intragenomic nrDNA ITS variability was examined in representative species of the *Stephanodiscus niagarae* complex. PCR was performed on DNA extracted from monoclonal cultures, and sequence variability determined using standard cloning techniques. Preliminary data from all species reveal intragenomic polymorphism. ITS sequence data is being used to delineate closely related species of algae and polymorphisms are potentially problematic for phylogenetic reconstruction and identity. We are exploring the extent of polymorphism in other freshwater Thalassiosiraceae, including *Stephanodiscus hantzschii* fo. *tenuis* and *Cyclostephanos invisitatus*.

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**SYSTEMATICS OF ULVACEAE (ULVOPHYCEAE, CHLOROPHYTA) USING NUCLEAR AND CHLOROPLAST DNA SEQUENCES**

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Ulvaceae are morphologically simple, cosmopolitan green macroalgae. Family members, particularly species of *Ulva* and *Enteromorpha*, are prevalent in coastal ecosystems and responsible for "green tide" events around the world. Little is known about the evolutionary history of Ulvaceae. A lack of reliable, non-molecular systematic characters has thwarted attempts to diagnose natural groupings within *Ulva* and *Enteromorpha* and among these and other putative Ulvaceae genera. Our study addresses several questions: 1) What are the phylogenetic relationships among genera commonly placed in Ulvaceae (*Chloropelta*, *Enteromorpha*, *Ulva*, *Ulvaria*); 2) Where do problematic genera such as *Blidingia*, *Capsosiphon*, *Korrmannia*, and *Percursaria* belong; and 3) What are the natural groupings of taxa within these genera? We address these questions using nuclear and chloroplast DNA sequences. Phylogenetic analyses support a monophyletic Ulvaceae consisting of *Chloropelta*, *Enteromorpha*, *Percursaria*, *Ulva* and *Ulvaria*. The systematic position of *Blidingia*, *Capsosiphon* and *Korrmannia*, and evolutionary relationships within selected genera will be discussed.

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**BIOLUMINESCENCE IN THE MARINE DINOFLAGELLATE *PYROCYSTIS LUNULA***

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Marine dinoflagellates are the main contributors to a phenomenon commonly known as "phosphorescence of the sea". Several investigators using different dinoflagellate species have shown that dinoflagellate bioluminescence is regulated by a circadian rhythm. Diverse bioluminescent systems exist in different organisms; however, all systems utilize an enzyme luciferase and the substrates luciferins, which show inter-species cross-reactivity. In marine dinoflagellates, luciferase and luciferin are localized in specific organelles, termed scintillons, which are usually located in close proximity to the vacuole during their active state. Bioluminescence is stimulated by mechanical stress or by acidification of the medium. Nicolas and co-workers (1987, *J. Cell Biol.* 105: 723-735) suggested that bioluminescence is triggered by an acidification of the scintillon caused by events that depolarize the tonoplast (proton trigger model). However, little is known about the signal transduction cascade that relays the mechanical stimulus perceived at the plasma membrane to the tonoplast. This study investigates the response of the bioluminescent system of *Pyrocystis lunula* to various parameters such as age of culture, light quality and quantity, disturbance (handling), sensitivity to chemicals, etc. to determine, if the bioluminescent response of *P. lunula* suitable for measuring environmental toxicants. The assay system used measures bioluminescence in response to mechanical stimuli or acidification allowing us to determine, if the response varies amongst the two different types of stimulation under different experimental conditions. The use of cell-permeant inhibitors for certain signaling pathways in this assay may help to biochemically characterize events involved in signal transduction from the plasma membrane to the scintillon.

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**REPRODUCTIVE, CULTURE AND REGENERATION STUDIES OF THE EDIBLE RED ALGA *CALLOPHYLLIS* IN CHILE**

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In Chile, the demand of edible seaweeds has increased during recent years and *Callophyllis variegata*

is one of the most demanded species. This study summarizes information on phenology, aspects, in vitro culture and vegetative propagation methods for *Callophyllis*. Results indicate that spore production occurs mainly during winter, and recruitment of new plants appear in nature in the spring. Culture studies indicate that spores presented higher germination and growth rates at 8° C and 10 to 12  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . Furthermore, these results indicate that this species presents a high potential for regeneration from its holdfast. The manipulation of temperature, light and culture medium enhances the regeneration process and growth of *Callophyllis* in the laboratory.

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### PHYLOGENY OF ZYGNEMOPHYCEAE BASED ON COXIII GENE SEQUENCE DATA

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Molecular phylogenetic analysis of the conjugating green algae (Class Zygnemophyceae) using nuclear (SSU rDNA) and chloroplast (*rbcL*) gene sequences has resolved hypotheses of relationship at the class, order, and family levels, but several key questions will require data from additional genes. Based on SSU and *rbcL* sequences, the Zygnemophyceae and Desmidiaceae are monophyletic, and families of placoderm desmids are distinct clades (Desmidiaceae, Peniaceae, Closteriaceae, and Gonatozygaceae). In contrast, the Zygnemataceae and Mesotaeniaceae are paraphyletic, although whether these two traditional families constitute a clade is uncertain. In addition, relationships of genera within families have proven resistant to resolution with these two oft-used genes. We have sequenced the *coxIII* gene from the mitochondrial genome to address some of these ambiguous portions of the phylogeny of conjugating green algae. The *coxIII* gene is more variable than *rbcL* or SSU rDNA and offers greater resolving power for relationships of genera. We present preliminary analyses of *coxIII* sequences from each of the traditional families of Zygnemophyceae and contrast the resulting topologies with those derived from nuclear and chloroplast genes.

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### CLONING AND CHARACTERIZATION OF NITRATE TRANSPORTER GENES FROM THE DIATOM *CYLINDROTHECA FUSIFORMIS*

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We have cloned and characterized the first nitrate transporter genes in a marine organism, from the diatom *Cylindrotheca fusiformis*. A cDNA clone and two genomic sequences, NAT1 and NAT2, were isolated. The sequences were highly conserved, with only 9 out of 484 amino acids differing. At least four copies of NAT genes were present in *C. fusiformis*, and multiple copies were present in other diatom species. NAT transcript abundance was high in cultures grown in the presence of nitrate, at nearly the same level during nitrogen starvation, and also high in urea-grown cultures. Lower mRNA levels occurred in nitrite-grown cultures. Transcript levels were highly repressed with  $\text{NH}_4\text{Cl}$  or  $\text{NH}_4\text{NO}_3$  as the nitrogen source, although very low amounts were detected. NAT mRNA levels were not directly regulated by light or dark. NAT mRNA levels varied, suggesting that nitrate uptake was not continuous, during progression through the cell cycle. The results suggest that transcriptional control contributes significantly to the overall control of nitrate uptake in *C. fusiformis*. The cloning of the nitrate transporter genes provides a new tool for investigating diatom nitrogen uptake and metabolism. In addition, the regulation of NAT expression by nitrogen source is likely to be useful in developing techniques to specifically control the expression of genes fused to NAT regulatory sequences in transgenic diatoms.

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### A MOLECULAR MOTOR FOR GLIDING MOTILITY IN CYANOBACTERIA

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Motile microorganisms either swim, by using flagella or glide over surfaces by mechanisms that are poorly understood. In cyanobacteria, gliding motility appears as a relatively slow and smooth surface-associated translocation in the direction of the long axis of the filaments at rates up to a few micrometers a second. Many filamentous species translocate in a highly coordinated manner. Translational movements are usually accompanied by revolutions around the long axis of the filament. While moving, the cyanobacteria secrete slime which is left behind as a twisted and collapsed thin tube. The observation of the slime secretion process shows that the mucilage is formed as fine bands that emerge in close proximity to the cells cross walls. Ultrastructural studies have revealed that the cyanobacteria possess at their cross walls complex, pore-like organelles, which might be involved in slime secretion. As each cell possess two different sets of pores

pointing in opposite direction, the coordinated activity of these structures could explain how the filament can reverse the direction of locomotion. Furthermore, ultrastructural studies have shown that rotating cyanobacteria possess cell surfaces formed by parallel, helically arranged surface fibrils. As the arrangement of these fibrils corresponds with the path of the filaments during locomotion, it might be imaginable that these fibrils serve as screw thread guiding the rotation of the filaments, with the necessary thrust for locomotion being derived from the secretion of slime using the pores at the cross walls.

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**EXPERIMENTAL STUDY OF PHYTOPLANKTON SPECIES COMPOSITION, ALTERNATE STATES, AND COMMUNITY BREAKPOINTS ALONG A PHOSPHORUS GRADIENT**

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A model combining algal mortality due to selective zooplankton grazing with the Monod model of phytoplankton growth was used to predict phytoplankton species composition along a gradient of phosphorus (P) concentration and zooplankton grazing pressure. Model predictions were compared to the results of a mesocosm experiment of phytoplankton species composition along a range of P concentrations of five to 115  $\mu\text{g L}^{-1}$  and potential grazing loss rates of 0.001 to 0.27  $\text{day}^{-1}$ . The effect of the P gradient was measured by monitoring phytoplankton and zooplankton species composition and biomass, and physical and chemical parameters for seven weeks in 12 fiberglass tanks (5500 L) filled with lake water and associated plankton. P concentrations were manipulated so that tanks were evenly distributed along the gradient. Using laboratory derived Monod growth constants for P and literature values of phytoplankton selectivity coefficients, zooplankton filtering rates, and zooplankton assimilation efficiencies, the model correctly predicted (1) the existence of alternate community states, (2) breakpoints between the alternate states which are similar to the P concentrations defining Vollenweider's lake trophic states, and (3) phytoplankton species composition along the P gradient in the mesocosm study. These results suggest that the trade-off between competitive ability for P and edibility is a primary factor structuring phytoplankton communities, and that multiple states exist which may be consistent with the long-standing P-based lake trophic classification.

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**SIGNIFICANCE OF PHYLOGENETIC SYSTEMATICS FOR THE BIOGEOGRAPHY OF THE MARINE RED ALGAE**

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Hovencamp (1997) recommends that geographical information can be extracted from cladistic analyses of phylogenetic data in which the Earth's history is resolved in terms of vicariance events that established barriers to migration. Nodes in a cladogram that specify two sister groups which do not overlap in their distributions are taken as evidence for a vicariance event and the sequential order of cladogram nodes leads to a procedure whereby the sequence of vicariance events can be reconstructed. For red algae, two such events are the persistence of the northward extension of the eastern end of Gondwanaland across a cool to warm temperature gradient with the formation of present-day Australasia, and the opening of the Tethyan Ocean followed by closure of the Tethyan Seaway between Africa and Eurasia. Phylogenetic hypotheses related to the first of these events are seen among genera belonging to the Bonnemaisoniaceae, Gracilariaceae, Kallymeniaceae, Gigartinaceae, and Delesseriaceae. A Tethyan origin and distribution is exemplified in part by families that comprise the Solieriaceae complex. Orders such as the Rhodymeniales, Halymeniales and the families Ceramiaceae and Rhodomelaceae contain taxa that fall partly into the first and partly into the second category. Phylogenies are constructed from *rbcL* sequence data and compared to the morphological evidence. The biogeographical speculations resulting from these observations are preliminary in nature and can only be confirmed or refuted with additional data and more refined analytical techniques.

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**THE COLONIAL GREEN ALGA *ASTREPHOMENE GUBERNACULIFERA* DISPLAYS A CHEMORESPONSE TO ACETATE**

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*Astrephomene gubernaculifera* is a colonial green alga capable of growth on sodium acetate in the dark. Because heterotrophic organisms often have mecha-

nisms to detect and respond to food sources, we tested the response of *A. gubernaculifera* to a number of potential chemoeffectors. Acetate elicits strong chemoaccumulation in three different behavioral assays. However, there was no chemoaccumulation in response to the carbohydrates xylose, ribose, glucose, galactose, mannose, arabinose, fructose, maltose, lactose, and sucrose; the amino acids asparagine, arginine, cysteine, glutamine, glycine, histidine, isoleucine, lysine, phenylalanine, serine, threonine, and hydroxyproline; or glycerol. Interestingly, *A. gubernaculifera* did respond to propionate. This compound is structurally related to acetate, but is incapable of supporting growth in the dark. This implies that the chemosensory response in *A. gubernaculifera* is not the direct result of metabolic changes but rather involves a specific chemical receptor and subsequent internal signaling pathway. We are presently analyzing swimming patterns in colonies exposed to changes in acetate concentration to test several alternate models that could lead to chemoaccumulation.

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#### ALGAL-RELATED TASTES AND ODORS IN PHOENIX WATER SUPPLY: PRELIMINARY REPORT

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Frequent episodes of algal-related tastes and odors (T & O) in drinking waters in metropolitan Phoenix, Arizona prompted initiation of a three-year project in July 1999 to investigate the occurrence of T & O metabolites and to develop a comprehensive management strategy to reduce the problems in drinking water supplies in arid environments. Two metabolites, 2-methylisoborneol (MIB) and geosmin, have been identified as compounds responsible for the earthy-musty tastes and odors in water supplies. Both were detected in the water supply system, including source rivers, reservoirs, canal delivery system and water treatment plants. Higher concentrations of MIB and geosmin occurred in distribution canals than in the upstream reservoirs indicating that significant production of the T & O compounds occurs within the canal system. A baseline-monitoring program has been established for the complex water supply system, with special emphasis on the canal system. Efforts are underway to investigate possible correlations between physical/chemical parameters, algal composition and biomass, with the occurrence of MIB and geosmin. In addition, several physical and chemical treatments are

planned for the canal system to reduce algal growth and related MIB and geosmin concentrations.

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#### A SYSTEMATIC STUDY OF GIGARTINACEAE FROM PACIFIC NORTH AMERICA BASED ON MOLECULAR AND MORPHOLOGICAL EVIDENCE

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Greater than 50 species of Gigartinaceae have been described from Pacific North America, about half of which are currently recognized. Although the family is treated extensively in the taxonomic literature, many of the species are still confused and a comprehensive revision is required. We sequenced the *rbdL* (RuBisCO) gene and ITS (Internal Transcribed Spacer) 1, 2, and 5.8S regions from a large number of recent collections and identified a discrete number of data sets. These were analysed in comparison with the morphological evidence for each of the taxa. Uncertain of the possibility that our operational taxonomic units may not correspond to the types, we developed a protocol for isolating PCR-friendly DNA from herbarium specimens, some reaching back as far as 1670. The DNA profiles of types and historically important specimens were compared to those for recently collected silica gel-dried and formalin-fixed material and assigned correct names. Species studied ranged from Alaska to Mexico and the Gulf of California and were compared to outgroup taxa from Pacific South America and the Southern Ocean. Particular attention was paid to variations in morphology as they relate to habitat with emphasis on the presence or absence of different morphological forms among sympatric and allopatric populations. We recognize 10 species in *Chondracanthus* (including one new combination and one new species) and 16 species in *Mazzaella* (including two new combinations and two new species). Finally, we tested a phylogenetic hypothesis inferred for the Gigartinaceae from *rbdL* sequences for congruence with one generated from ITS sequences.

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#### QUALITATIVE AND QUANTITATIVE STUDIES OF THE SWIMMING BEHAVIOR OF *HINCKSIA IRREGULARIS* SPORES (PHAEOPHYCEAE)

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The swimming behavior of spores of the brown alga *Hinckesia irregularis* was analyzed using computer-assisted



motion analysis. We distinguished five main swimming patterns: straight paths, search circles, orientation, gyration, and wobbling. We suggest different functional values for the individual swimming patterns. Straight paths, search circles, and orientation are different but all may be important in small-scale movements in the benthic boundary layer. As such, they could enable a spore to find a suitable microenvironment for germination and growth. Gyration occurs during the initial reversible phase of adhesion that can lead to settlement. Wobbling is typical for irritated or mechanically damaged spores and does not seem to be a typical pattern associated to settlement. The dominant swimming patterns changed with spore age ( $10 \pm 5$  to  $60 \pm 5$  min of spore age), with young spores mainly swimming in straight paths and search circles and older spores in orientation and gyration. This change in swimming patterns can be quantified by speed (decrease over time) and rate of change of direction (increase over time). Based on these results, we suggest that computer-assisted motion analysis of the swimming behavior of *H. irregularis* spores can be used to develop bioassays with both ecological and technological relevance.

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**A NOVEL BIOFOULING ASSAY USING COMPUTER-ASSISTED MOTION ANALYSIS OF *HINCKSIA IRREGULARIS* SPORE SWIMMING**

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Computer-assisted motion analysis of the swimming behavior of spores of the brown alga *Hinckesia irregularis* was used in a novel antifouling bioassay. Newly released spores were exposed to water soluble compounds of organic and aqueous body wall extracts of two sympatric sea stars, *Astropecten articulatus* and *Luidia clathrata*. Changes in the swimming behavior of *H. irregularis* spores were quantified by changes in speed (decrease at extract exposure) and rate of change of direction (increase at extract exposure). Sea star extracts were tested at natural concentrations (mg extract/g wet weight) and at approximate 10-, 100-, and 1000-fold dilution. Significant effects ( $p \leq 0.05$ ) were observed at 1000-fold dilution of the natural concentration in both extracts of *A. articulatus*, and at 100- and 10-fold dilution of the aqueous and organic extract, respectively, in *L. clathrata*. Germination of spores after short time exposure (30 min) to sea star body wall extracts indicates that compounds were not toxic but affected spore mobility, a factor that plays an important role during spore settlement. We suggest that this assay is very useful for bioassay-guided fractionation of extracts because it is very sensitive and requires only small amounts of extracts or isolated compounds.

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**ENDOMEMBRANE ULTRASTRUCTURE AND POSSIBLE CHLOROPLAST PROTEIN IMPORT PATHWAY IN *HETEROSIGMA AKASHIWO* (RAPHIDOPHYCEAE)**

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Chloroplasts in heterokont algae probably originated from a red algal endosymbiont which was engulfed and retained by a eukaryotic host, and are surrounded by four envelope membranes. The outermost of these membranes is called chloroplast ER (CER) and usually connects with the nuclear envelope. This information, however, is based mainly on studies on single-plastid heterokont algae. In multi-plastid heterokont algae, it is still unclear whether CER is continuous with the nuclear envelope. Since nuclear-encoded chloroplast proteins are synthesized by ribosomes on the ER membrane, clarifying the ER-CER structure in the heterokont algae is important in order to know the targeting pathway of those proteins. We did a detailed ultrastructural observation of endomembrane systems in a multi-plastid heterokont alga: *Heterosigma akashiwo*, and confirmed that the CER membrane was continuous with the ER membrane. However, unlike the CER membranes in other heterokont algae, it seemed to have very few ribosome attached. We also performed experiments for protein targeting into canine microsomes using a precursor for a nuclear-encoded chloroplast protein, a fucoxanthin-chlorophyll protein (FCP), of *H. akashiwo*, to see if the protein is targeted to the ER. It demonstrated that the precursor has a functional signal sequence for ER targeting, and is co-translationally translocated into the microsomes. Based on these data, we propose a hypothesis that, in *H. akashiwo*, nuclear-encoded chloroplast protein precursors that have been co-translationally inserted into the ER lumen are sorted in the ER and transported to the chloroplasts through the ER.

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**ENHANCED GROWTH RATES OF THE GERMINGS OF CYANOBACTERIAL RESTING SPORES (AKINETES)**

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In experiments with the toxic cyanobacterium, *Anabaena circinalis*, we investigated the nature of cyst formation and germination under different environmental conditions (variation in light quality and quantity,

temperature, nutrient limitation). We consistently found that germlings up to about 5 days old had greater growth rates than those of vegetative cells grown under similar saturating irradiance and excess nutrient conditions in batch culture. Averaging the results from 4 experiments, the specific growth rate ( $K'$ ) fell from 1.9 d<sup>-1</sup>, 24 hours after germination to 1.17, 1.04, 0.8, and to 0.45 d<sup>-1</sup> after 48, 72, 120 and 144 hours, respectively. This equates to an increase in generation time from 0.37 to 1.54 days over this 6 day period. To investigate the implications of these results we modelled two hypothetical populations, A & B, with an initial akinete or resting cyst concentration of 10/mL each, but with population A having a standard growth rate of 0.45 and B with the 6 day early enhanced growth rate as determined in our laboratory experiments. The model indicates that population B quickly establishes dominance over population A. In this modeling exercise we have assumed a germination frequency of 100% for both populations but our laboratory results indicate that even given optimum germination conditions this factor may vary between 5% and 90% depending on the age of akinetes and the conditions experienced during dormancy (senescence). Some conditions that determine germination frequency were also investigated.

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### RELATIONSHIP BETWEEN PERIODIC RESUSPENSION EVENTS AND PHYTO-PLANKTON BLOOMS IN LAKE MICHIGAN

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Lake Michigan provides an ideal location for comparing episodic physical forcing effects (storm events) on phytoplankton processes and the more persistent seasonal variability of phytoplankton communities. This is because of the duration and extent of the highly turbid, recurrent coastal plume (RCP) in the lake during the winter/early spring. Although the RCP coincides with the initiation of the basin wide spring diatom bloom, linkages between the duration and intensity of the plume and the prominent role of light availability in regulating Lake Michigan phytoplankton growth during the spring isothermal period have been postulated, but not verified. As such the concurrent physical and biological events provide a novel opportunity to examine how variations in biological rate processes, coupled with transport and resuspension phenomena associated with the RCP, affect the distributions of organisms, evolution of communities, and growth and primary production of Lake Michigan phytoplankton. In this study, phytoplankton assemblages from pre, post, and active plume events are ex-

amined from three stations in Lake Michigan. Species assemblages include chlorophytes, chrysophytes, and diatoms. The diatom flora was particularly abundant. Species composition changed rapidly during storm events, with resting cell forming taxa playing a significant role in these modifications. Ephemeral species appeared during the circulation event and decreased once storm activity subsided. Other taxa were able to establish themselves in the phytoplankton community during the plume events and persisted after storm activity subsided. Understanding activity during these circulation events will lead to a better understanding of overall community structure development throughout the year.

## 103

### PHYLOGENETIC ANALYSIS OF THE MARINE AND FRESHWATER THALASSIOSIROID DIATOMS

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The thalassiosiroid centric diatoms are distinguished by at least one synapomorphy, the strutted process or fulcrotortula. Various classified as a family (Thalassiosiraceae) or an order (Thalassiosirales) among centric diatoms, it is generally conceded that the group of several hundred fossil and living species is monophyletic as a whole. There are two ecological groups of thalassiosiroids, marine and freshwater. It has been hypothesized, based on an eclectic, non-rigorous, evolutionary taxonomy perspective that both the marine and freshwater ecological groups are also monophyletic, but this hypothesis has never been tested in a rigorous framework. Likewise, the freshwater thalassiosiroid species have been grouped into several genera and subgenera using an evolutionary taxonomic approach, but these hypotheses have not fully been tested using cladistic analysis. Focusing mainly on freshwater species, but including at least one representative of each marine genus and one representative from each of several proposed subgeneric groupings of the genus *Thalassiosira*, we scored morphological characters for fossil and living marine and freshwater Thalassiosiraceae to test these hypotheses. Our cladistic results provide strong support for monophyly for the freshwater group, but it seems unlikely that the marine group is monophyletic. The cladistic results are corroborated to greater or lesser degrees by the fossil record. The implications for evolution in the group and for taxon sampling in molecular studies we are conducting will be discussed.

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**PHYLOGENY OF THE BASAL LINEAGES OF STREPTOPHYTA BASED ON *RBCL* AND *ATPB* GENE SEQUENCE DATA**

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The streptophytes comprise the Charophyceae *sensu* Mattox and Stewart (a morphologically diverse group of fresh-water green algae) and the embryophytes (land plants). Several charophycean groups are currently recognized. These include the Charales, Coleochaetales, Chlorokybales, Klebsormidiales and Zygnemophyceae (Desmidiales and Zygnematales). Recently, SSU rRNA gene sequence data allied *Mesostigma viride* (Prasinophyceae) with the Streptophyta. Complete chloroplast sequence data, however, placed *Mesostigma* sister to all green algae, not with the streptophytes. Several morphological, ultrastructural and biochemical features unite these lineages into a monophyletic group including embryophytes, but evolutionary relationships among the basal streptophytes remain ambiguous. To date, numerous studies using SSU rRNA gene sequences have yielded differing phylogenies with varying degrees of support dependent upon taxon sampling and choice of phylogenetic method. Like SSU data, chloroplast DNA sequence data have been used to examine relationships within the Charales, Coleochaetales, Zygnemophyceae and embryophytes. Representatives of all basal streptophyte lineages have not been examined using chloroplast data in a single analysis. Phylogenetic analyses were performed using DNA sequences of *rbcl* (the genes encoding the large subunit of rubisco) and *atpB* (the beta-subunit of ATPase) to examine relationships of basal streptophyte lineages. Preliminary analyses placed the branch leading to *Mesostigma* as the basal lineage in the Streptophyta with *Chlorokybus*, the sole representative of the Chlorokybales, branching next. Klebsormidiales and the enigmatic genus *Entransia* were sister taxa. Sister to these, the Charales, Coleochaetales, embryophytes and Zygnemophyceae formed a monophyletic group with Charales and Coleochaetales sister to each other and this clade sister to the embryophytes.

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**THE PINGUIOPHYCEAE CLASSIS NOVA, CHROMOPHYTE ALGAE PRODUCING LARGE AMOUNTS OF OMEGA-3 FATTY ACIDS**

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A new class of algae is proposed for a small group of unusual chromophytes (=heterokont algae). One novel aspect of these organisms is that cells contain large amounts of polyunsaturated fatty acids (41 – 67% of fatty acids), with exceptionally high amounts of EPA (21 – 56% of fatty acids). A second unusual character is that, for at least two genera, the mature flagellum (short, smooth flagellum of other chromophytes) is dominant, and the immature flagellum (long, hairy flagellum of other chromophytes) lacks tripartite hairs and is reduced in size or even absent. Carotenoid analysis shows that the class is characterized by the presence of chlorophylls *a* and *c*, fucoxanthin, violaxanthin, antheraxanthin, zeaxanthin, and  $\beta$ -carotene. The 18S rRNA and *rbcl* gene sequences show that these organisms form a monophyletic group that is supported by very high bootstrap values. The phylogenetic relationships of the Pinguiophyceae and other classes are not well resolved, however, the relationships among the chromophytes have always been uncertain. The abundance of EPA, known to reduce “bad cholesterol” in humans, suggests this new class may be of significant economic importance.

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**REGENERATION OF PROTOPLASTS FROM DISINTEGRATED CELLS OF *BRYOPSIS PLUMOSA* (DERBESIALES, CHLOROPHYTA)**

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When injured, the protoplasts come out from the multi-nucleate giant cell of a green alga *Bryopsis plumosa* and can generate numerous new cells spontaneously. The cell organelles aggregated rapidly in sea water and became covered with a gelatinous envelope within fifteen minutes. A lipid cell membrane was formed inside the envelope within nine to twelve hours. Cytochemical studies using Nile Red and various enzymes revealed that the primary envelope is initially composed of polysaccharides then becoming a polysaccharide-lipid complex. Fluorescein diacetate (FDA) staining showed that the primary envelope has some characteristics of cell membranes including semipermeability and selective transport of materials. The aggregation of cell organelles appears to be mediated by two kinds of material; one in vacuolar sap

and the other on the surface of cell organelles. About a thousand new cells were generated from a single disintegrated branch and forty percent of them eventually developed into mature plants.

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#### **PATTERNS OF RECRUITMENT AND DISTRIBUTION OF *ULVA* AND *ENTEROMORPHA* POPULATIONS IN A KOREAN INTERTIDAL SHORE**

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Patterns of recruitment and abundance of *Ulva pertusa* and *Enteromorpha linza* in the three locations on the south coast of Korea where green tide frequently occurred were investigated with respect to the substrate profile, temporal and spatial variations, and their persistence. Artificial substrates (10 to 20 cm) were made of a mixture of a piece of granite rock and cement on ceramic tiles. These setting plates, representing different surface texture, were placed horizontally and vertically along the rocky shoreline, and replaced every 1 to 2 months. Both populations showed highly seasonal blooms, with peaks in October, 1998 to February, 1999 and another slight peak was observed in the fall of 1999 only for *Ulva*. However, the winter peak of 1998 did not appear in the following year, indicating that a significant year-to-year variation existed. Regarding their settlement onto artificial plates with different roughness, *Ulva* preferred to settle on the rough surface both in the upper and lower intertidal zone; however, this pattern only appeared in the upper zone for *Enteromorpha*. Vertical distribution of the two populations was well separated; *Enteromorpha* was more abundant in the upper zone and *Ulva* in the lower zone. Their seasonal blooms were concomitant with ammonia levels, but occurrence was not well correlated with nitrate and phosphate. The ability of the populations to persist, once blooms occurred, was also distinct; *Ulva* persisted ca. twice as long as *Enteromorpha*, having a greater impact in the presence of green tide in this geographical area.

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#### **ULTRAVIOLET ABSORBING SUBSTANCES FROM *AGARUM FIMBRIATUM* (PHAEOPHYCEAE) PROTECT *GRIFFITHSIA PACIFICA* FROM UV RADIATION**

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We examined whether naturally occurring polyphenols produced by a brown alga can protect other organisms from ultraviolet radiation. Fragments of *Griffithsia pacifica* were grown in medium supplemented with a seawater extract of *Agarum fimbriatum* (3% w/v in seawater for 2 h). Cultures were exposed to cool-white fluorescent light (7–7.5  $\mu\text{Mol photons cm}^{-2} \text{s}^{-1}$ ) in the presence or absence of 130 to 168  $\mu\text{W cm}^{-2}$  UV-A and 85 to 112  $\mu\text{W cm}^{-2}$  UV-B. Regenerating fragments were scored for five qualitative or quantitative measures of growth. Rhizoid growth was not inhibited by the polyphenol extract after ten days. UV radiation in the absence of polyphenols caused considerable cell death and slower growth. Thus, the presence of polyphenols provided protection from UV radiation such that in the highest concentration of polyphenols (1:1 medium:extract), rhizoid growth in UV exposed fragments was approximately 60% as great as control plants without both polyphenols and UV radiation. Plants exposed to UV radiation in the absence of polyphenol extract showed poor rhizoid regeneration and extensive cell death. These experiments document the nature of UV-A and UV-B damage to growing red algae, and provide strong evidence that polyphenols from brown algae may have a role in protecting subtidal, benthic algal communities from UV radiation.

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#### **VERTICAL MIGRATION OF *EUGLENA* SP. ON THE SAND BANKS OF A NORTH CAROLINA PIEDMONT STREAM**

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On summer days, visible patches of green appeared on the aerially-exposed sand banks of a small tributary of Mays Lake in Burlington, North Carolina. These green patches became visible approximately 3 hours after sunrise and disappeared 2 hours prior to sunset. Microscopic examination of sediment samples revealed that the appearance of color was due to the upward migration of *Euglena* sp. that attained densities of 2200 cells per square millimeter around solar noon. Triplicate sediment cores revealed the presence of live *Euglena* cells as deep as 6 cm below the sediment surface. During the day, over 80% of the population was located between the surface and a depth of 2 mm. During the night, 75% of the population was found between 2 and 6 cm below the surface. Artificially darkening the sediment surface during the day stimulated downward migration of the popula-

tion. Controlled experiments with neutral density filters indicated that the number of cells on the sediment surface was directly related to incident irradiance. The results of these experiments on *Euglena* sp. living on the banks of a freshwater stream in central North Carolina will be contrasted with those of previous experiments conducted on *Euglena proxima* living on the intertidal sand flats of the Newport River estuary in coastal North Carolina.

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### **INFLUENCE OF ANTHROPOGENIC POLLUTION ON MACROPHYTOBENTHOS OF THE AVACHA BAY (SOUTHEAST KAMCHATKA, RUSSIA)**

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Most industrial and agricultural enterprises of the Kamchatsky district are situated along the coast of the Avacha Bay and its river drainage. Intensive water pollution resulted in extreme deterioration of the bay's ecology. The destruction of macrophytobenthos under the influence of pollution was investigated. Materials were regularly collected at the same sampling sites in different seasons of the year from 1988 up to 1999. Analysis of our data allowed us to determine directions of long-term changes of species composition and macrophytobenthos structure, speed of degradation of different types of vegetational communities and reasons for all these processes. The Avacha Bay's flora consisted of more than 160 species up to 1970s, including species belonged to 104 genera, 47 families, 23 orders, 3 divisions (30 Chlorophyta, 48 Phaeophyta, 82 Rhodophyta). Flora of the internal part of the bay differed from that of the bay's mouth and consisted of 143 and 146 species, respectively. Presently, the whole flora of the bay (internal part+throat) includes only 94 species. Along the urban coast 18 poly- and mesosaprobious species are found only. They are characterized by small sizes, low productivity and short life cycles. Most of them are ephemeral with a primitive morphology. The responsive reaction of algae to pollution resulted in decreases in size and weight of the thalli, and appearance of anomalies of development. *Laminaria bongardiana* was used as a bioindicator. The changes of weight and sizes of its plants allow us to determine degree of the pollution in different parts of the coast of the Avacha Bay.

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### **SEASONAL VARIATIONS IN PHYTOPLANKTON COMMUNITIES OF AN EPHEMERAL POND SYSTEM IN SOUTHEASTERN VIRGINIA**

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The Grafton Ponds Natural Area is a 151 hectare preserve in York County, Virginia, approximately 56km north of the City of Norfolk. The preserve contains over 40 ephemeral ponds. These ponds which are filled seasonally by precipitation and groundwater discharge, are typically wet from late fall through late spring or early summer. Pond size varies from 0.1 to 2 ha, and depths range from 4 cm to 3 meters. The first phase of this study was to examine the physical and chemical characteristics of five of these ponds, including pond size and depth, inundation period, water temperature, pH, and phosphate and ammonium concentrations. The second phase, which is currently underway, is to examine the phytoplankton assemblages, relating differences in the phytoplankton populations to the varying physical and chemical characteristics of the ponds. To date, analysis indicates Chlorophytes and Cyanobacteria as dominant groups in the winter and spring with diatoms becoming more abundant in the late summer and fall. Periodic Dinoflagellate blooms also occur. Analysis also indicates rapid turnover of species from month to month.

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### **FIELD STUDY OF ALGAL RECRUITMENT BY CLEARING EXPERIMENT IN PING CHAU, HONG KONG SAR, CHINA**

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Recruitment, the entry of new individuals into a population, was investigated by a clearing experiment along the shore of A Ma Wan (AMW) in Ping Chau, Hong Kong SAR, China. Two types of clearing, with all the existing vegetation removed (cleared) and with the top 2 to three mm of the rock surface removed (hammered), were carried out monthly in AMW from November 1997 to June 1999. Observations were made one month after clearing and on a monthly basis thereafter. The number of algal species present on the cleared areas and their percentage cover were recorded. The experimental results showed that more algal species were recruited during March and April in 1998 (n=10) but in 1999, the number of species was found higher in February and March (n=8). Species richness dropped after May (more obvious in 1998), indicating that recruitment greatly declined in summer. A tuft complex composed of several filamen-

tous algal species dominated over the others in all clearing areas (coverage mostly over 90%) but recruits of *Caulerpa peltata*, *Colpomenia sinuosa*, *Enteromorpha* sp., *Hypnea charoides*, *Padina* spp., *Sargassum* sp., *Spyridia filamentosa*, *Ulva* sp., etc. also were observed during the study period. Generally, there were no significant differences in terms of species richness and composition of the recruits between the two treatments (cleared vs. hammered) as well as with the controls. This implied that algae in AMW were more likely to be recruited de novo from elsewhere rather than regenerated from remnants of the previous year's growth.

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#### NUCLEAR RIBOSOMAL DNA PHYLOGENY OF HALIMEDALES REVEALS SEVERAL POLYPHYLETIC GENERA

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Halimedales (Chlorophyta) are widely distributed in reefs and lagoons throughout the tropics and subtropics. One genus, *Chlorodesmis*, consists of simple, branching siphons that persist into the mature sexual phase. In most other genera, only the juvenile stages exhibit such simple anatomy. Their mature, often-calci-fied thalli possess complex siphonal anatomy and differentiation depending on the location in the thal-lus. We obtained a nuclear ribosomal DNA phylogeny encompassing many genera in order to unravel the evolution of this complexity. Results pinpoint *Caul-erpa* as outgroup to a clade with all other genera included in this study. The first dichotomy separates *Halimeda* from the remaining genera. Distinct gamet-angial anatomy defines these two principal clades. Within the remainder, *Tydemania* and its sister *Flabel-lia* are nearest neighbors to a clade containing *Rhipo-cephalus*, *Udotea*, *Penicillus* and *Chlorodesmis*. The latter three genera are polyphyletic. Therefore, character states of thallus superstructure defining these genera appear to be homoplasies. *Chlorodesmis* represents a case of neoteny where the juvenile stage persists into reproductive maturity. We will evaluate which mor-phological characters do change state in agreement with the molecular phylogeny and can, therefore, serve as diagnostic characters for a more natural clas-sification.

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#### NITROGEN ASSIMILATION AND ALLOCA-TION STRATEGIES OF POLAR SEAWEEDES FROM DIFFERING NUTRIENT ENVIRON-MENTS

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Nitrogen assimilation and allocation strategies were compared in polar macroalgae from environments with similar near-freezing temperatures and low annual irradiance but differing dissolved inorganic nitrogen (DIN) regimes. The antarctic endemic, *Himan-tothallus grandifolius*, experiences high nitrate con-centrations year-round and occasionally high, but variable, ammonium levels. *H. grandifolius* cannot store nitrogen or maintain growth under periods of low N-supply. The arctic endemic, *Laminaria solidun-gula*, is exposed to seasonal fluctuations in DIN, with N-limitation occurring during the summer. *L. solidun-gula* showed "storage-specialist" characteristics of ni-trate uptake, with high *V*<sub>max</sub> allowing this species to take advantage of seasonally elevated nitrate concen-trations to accumulate large organic and inorganic N-reserves. *H. grandifolius* had a high *V*<sub>max</sub> for ammo-nium, allowing the alga to utilize pulses of this nutri-ent. In the presence of both DIN forms, nitrate up-take was significantly reduced in both species. Furthermore, *H. grandifolius* and *L. solidungula* dem-onstrated significantly reduced uptake and assimila-tion of nitrate during short-term and prolonged peri-ods of darkness, while ammonium uptake and as-similation were relatively unaffected by light. Al-though preferential uptake of ammonium, particu-larly in the dark, allows both species to conserve en-ergy in their cold, low-light environments, the antarctic species, which does not have the additional problem of N-limitation, showed stronger energy con-serving traits. Nitrogen assimilation characteristics of the arctic species appeared to balance energy con-servation with the need to minimize N-limitation in an environment that alternates between low light and low N availability.

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#### EXPRESSION PATTERNS OF THREE PUTA-TIVE SEXUAL-SPECIFIC GENES IN THE CEN-TRIC DIATOM *THALASSIOSIRA WEISSFLOGII*

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An intriguing feature of the diatom life cycle is that sexual reproduction not only generates genetic diver-sity but also controls cell size. Very little is known, however, about either the frequency or the potential ramifications of sexual events in field populations, due in large part to the difficulties associated with rec-ognizing sexual stages. Our approach has been to

identify genes involved in the diatom sexual cycle, with the goal of developing molecular probes to determine when and where sexual reproduction occurs. We are investigating the expression pattern of three genes identified in a sexual reproduction screen. The Sig1, Sig2 and Sig3 genes comprise a gene family, and encode proteins with strong sequence homology to extracellular matrix proteins. We have hypothesized that the Sig proteins are necessary for sperm/egg recognition. Using RT-PCR, we have shown that transcription of the Sig genes is highly upregulated during the early stages of the sexual cycle, before any morphological changes are apparent. The three genes show significant differences in the exact timing and levels of expression, suggesting different roles for each member of the gene family. We are now employing quantitative RT-PCR to study their relative transcription levels in more detail. We are also using antibodies made against a recombinant version of the Sig2 protein to look at protein expression levels before and after sexual induction, and to determine where the Sig2 protein localizes in the cell.

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##### **PRELIMINARY EVALUATION OF THE BIOREMEDIATION POTENTIAL OF *PORPHYRA*: PHOTOSYNTHETIC PRODUCTION BY BLADES AND *CONCHOCELIS***

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Given their rapid growth and nutrient assimilation rates, *Porphyra* spp. are good candidates for bioremediation. The production potential of two northeast U.S. *Porphyra* species currently in culture (*P. purpurea* and *P. umbilicalis*) was evaluated by measuring rates of photosynthesis (as O<sub>2</sub> evolution) of samples grown at 20° C. Gametophytes of *P. umbilicalis* photosynthesized at rates that were 80% higher than those of *P. purpurea* over 5–20° C at both sub-saturating and saturating irradiances (37 and 289 μmol photons m<sup>-2</sup> s<sup>-1</sup>). *Porphyra umbilicalis* was both more efficient at low irradiances (higher alpha) and had a higher P<sub>max</sub> than did *P. purpurea* (23.0 vs. 15.6 μmol O<sub>2</sub> g<sup>-1</sup> DW min<sup>-1</sup>), suggesting that *P. umbilicalis* is a better choice for mass culture where self-shading may be severe. The photosynthesis-irradiance relationship for the *Conchoceleis* stage of *P. purpurea* was also examined. Tufts of filaments, grown at 10, 15, and 20° C, were assayed at growth temperatures at irradiances ranging from 0–315 μmol photons m<sup>-2</sup> s<sup>-1</sup>. Tufts were slightly more productive at 15° than at 10° C, but only ca. 4–6% as productive as gametophytes. Maximum rates of net photosynthesis were reduced by 66–74% in tufts

grown at 20° C (only about 2% of gametophytes). The *Conchoceleis* stage, however, need not limit mariculture operations; once *Conchoceleis* cultures are established, they can be maintained over the long-term as ready sources of spores for net seeding.

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##### **NEW GENERA OF FRESHWATER CRYPTOMONADS FROM COLORADO**

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Electron microscopic examinations of numerous isolates from Colorado lakes and reservoirs have revealed several new genera and species. Based on the furrow/gullet variations and the structure of the periplast, four new genera are proposed. These new genera and their characteristics are as follows. The first genus has a furrow without a gullet, and it has oval periplast plates. There are two chloroplasts per cell and two nucleomorphs located anterior to the chloroplasts in the periplastidial compartment. Pyrenoids are lacking. Chloroplasts contain phycoerythrin 566. The second genus is similar in shape and structure to *Plagioselmis*, but it has small rectangular plates rather than hexagonal plates. There is one dorsal chloroplast per cell, with a single stalked pyrenoid, which is not traversed by thylakoids and associated with two starch plates. The nucleomorph is posterior to the pyrenoid and is located between the nucleus and pyrenoid. Chloroplasts contain phycoerythrin 566. The third genus lacks a gullet and furrow and has an inner periplast sheet. Cells have two chloroplasts without pyrenoids, and the chloroplasts contain phycoerythrin 566. The nucleomorphs are located in the anterior portion of the plastidial compartment. The fourth genus is blue-green in color, it lacks a furrow/gullet, and the internal and surface periplast components consist of hexagonal plates. Cells have a large pyrenoid that protrudes ventrally into the cytoplasm and is traversed by numerous paired thylakoids. The nucleomorph is situated lateral to the pyrenoid. Chloroplasts contain phycocyanin 645. This cryptomonad was previously identified as *Chroomonas pochmanni*, and it is mixotrophic.

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##### **GENETIC STRUCTURE VARIABILITY OF KELP *ALARIA MARGINATA* OVER SPACE AND TIME**

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The genetic variability of *Alaria marginata* Postels & Ruprecht was investigated spatially and seasonally using the fingerprinting technique of amplified fragment length polymorphism (AFLP). Using 206 scoreable bands generated by one primer pair, individual plants that were separated by as little as a few decimeters to >100 km could be distinguished, and followed an isolation-by-distance model. Genetic similarity ranged from 76% for patches (a few decimeters in diameter), to 71% for individual kelp stands (15 m across) and 67% for a group of stands separated by 185 km. Greater genetic similarity of patches occurred at the wave-sheltered site than at wave-exposed site. The influence of wave to genetic variability and the ability to predict gene flow on small stretch of beach were discussed. In one stand, genetic similarities were markedly different between seasons. This seasonal pattern may be the result of different age groups dominating the sampled stands over time. The genetic structure of *A. marginata* comprises local scale (patch and within stand) heterogeneity and larger scale (between stands) homogeneity.

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#### **MOLECULAR ANALYSES OF LINEAR CHLOROPLAST PLASMIDS FROM *ERNODESMIS VERTICILLATA***

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*Ernodesmis verticillata* contains novel, linear plasmid-like DNA molecules in its chloroplasts, whose function remains unclear. Their molecular architecture is putatively a "hairpin," wherein every molecule consists of a long inverted repeat folded back on itself. Thus, each molecule is composed of a terminal (telomeric) domain, a central inverted repeat, and a "loop" domain. Cloning strategies have been devised for characterizing the terminal and loop regions, since they might contain landmark features like replication origins. Polymerase chain reaction (PCR) was used to amplify loop domains of native molecules, and ligation of the PCR products with commercial cloning vectors initially yielded 11 clones. So far, no recognizable sequences have turned up in the loop domains of the molecules. Unlike what has been reported for most linear plasmids, we have been unable to verify that any proteins are associated with either the 5'- or 3'-ends of the *Ernodesmis* plasmids. In fact, the 5'-end of each molecule contains a terminal phosphatase

that is accessible to alkaline phosphatase and subsequently to T4 polynucleotide kinase *in vitro*. It is also possible to modify the 3'-end with terminal deoxynucleotidyl transferase (TdT) for homopolymeric tailing. Poly-(C) tailing of native molecules promotes their annealing to poly-(G) tailed vectors, for cloning of the terminal domains. An initial library of 14 TdT clones (10 unique) indicates that short (11–28 bp) direct repeats occur near the termini of the plasmids. Shorter (4–6 bp) inverted repeats at the very ends may lead to terminal foldbacks that might serve to protect the termini.

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#### **LIFE ON THE EDGE: STRESS SURVIVAL ADAPTATIONS IN SOUTHERN LIMIT *MACROCYSTIS PYRIFERA* POPULATIONS**

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In spite of subtropical temperatures often occurring at the *Macrocystis pyrifera* distributional southern limit in the Northern Hemisphere in Baja California, Mexico, the population has been successful at surviving and recovering from disturbance. To explore the physiological characteristics of southern limit *M. pyrifera*, microscopic and macroscopic phases of southern limit plants were tested for stress tolerance and compared to northern plant responses in three experiments. In spore development experiments, under constant temperature and nitrate stress treatments, spores from the southern-limit population survived higher temperatures, had greater survivorship, and were able to complete their life cycle at higher temperatures and lower nitrate concentrations than northern population spores. During stress-recovery treatments, where all microscopic stages were tested for recovery after stress shock exposure (temperature, nitrate and light stress), 96% of all haploid treatments showed some regrowth during recovery; haploid survival was not significantly affected by spore population origin, light, or nitrate. However, only 26% of diploid treatments showed regrowth and diploid survival was significantly affected by spore population origin; only the southern limit spores recovered after stress treatment exposure. In juvenile transplant experiments, under all conditions, juvenile plants originating from the southern-limit had higher tissue nitrogen content, greater frond addition rates, and higher photosynthetic efficiency than northern plants, particularly at higher temperatures. The physiological differences observed for the southern limit plants versus those from the north contributes to our understanding of how the southern limit plants can survive in an environment that might otherwise be considered extreme for the species.



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### ACCUMULATION OF DOMOIC ACID BY THE COASTAL DIATOM *PSEUDO-NITZSCHIA MULTISERIES*: A POSSIBLE COPPER COMPLEXATION STRATEGY

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Domoic acid (DA) is a neurotoxic amino acid produced by several members of the diatom genus *Pseudo-nitzschia*. Trophic transfer of DA up the food chain has been implicated in the deaths of 100's of marine birds and marine mammals along the central California Coast. The physiological function of DA in *Pseudo-nitzschia* spp. has not been defined, although some evidence indicates that elevated metal concentrations can induce DA accumulation (Subba RAO *et al.*, 1998, P.S.Z.N. *Mar. Ecol.* 19:31). Although California coastal waters have experienced a decline in several heavy metals from 1977–1990, copper concentrations have increased by as much as 25% (Stephenson, M. D. & Leonard, G. H., 1994, *Mar. Poll. Bull.* 28:148). Many algae produce chelators, including amino acids, in response to toxic  $[Cu^{2+}]$  (Wu *et al.* 1998, *J. Phycol.* 34: 113). Domoic acid, a tricarboxylic acid, has 4 functional groups that may readily form chelation complexes with transition metals like copper. Copper enrichment experiments indicate that while  $Cu^{2+}$  is toxic to *Pseudo-nitzschia multiseries* at total  $[Cu]$  greater than  $16.1\mu M$  (pCu 6.0), intracellular DA accumulation increases up to this point with no decline in growth rates relative to cultures grown in standard enriched seawater. These data suggest that DA may be accumulated by *P. multiseries* to mitigate the toxicity of elevated  $[Cu^{2+}]$ . Chemiluminescence will be used to quantify the binding affinity (expressed as conditional stability constants,  $K_c$ ) of DA for  $Cu^{2+}$ . Defining the Cu-DA dose response relationship in *Pseudo-nitzschia* can facilitate prediction of future toxic bloom events.

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### THE UBIQUITOUS PRESENCE OF CHLOROPHYLL *D* IN RED ALGAE FROM THE MONTEREY PENINSULA

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The presence of chlorophyll *d* in red algae has been a subject of great controversy, however, only a few studies have been devoted to this subject, and evidence for its existence has been inconsistent. Here we report on results of a survey of 56 species of red macroalgae from central California, 3 species from Baja California Sur, Mexico, and 2 species from Cape Evans, Antarctica. Chlorophyll *d* was detected in 48 of the California species, 1 of the Mexico species, and 1 of the Ant-

arctica species. Chlorophyll *a/d* ratio ranged from 6 to 21123. The fraction of species of red algae containing chlorophyll *d* was higher in samples collected in October (36 out of 36), than those collected in June (12 out of 29) or March (4 out of 22). Chlorophyll *d* was not detected in any of the brown or green macroalgae analyzed. *In vivo* absorption spectra of *Gelidium* sp. were collected, and resolution of these spectra by fourth derivative resulted an absorption maximum at 687 nm due to chlorophyll *a*, and at 719 nm due to chlorophyll *d*. Extracted pigment of *Gelidium* sp. in 90% acetone had an absorption maximum of 664 nm, and a smaller absorption peak at 692 nm. Fluorescence emission and excitation spectra resulted in a maximum of 699 nm and 447 nm, respectively. The emission peak wavelength of 699 and an absorption peak longer than that of chlorophyll *a* (699 nm compared to 664 nm) suggest chlorophyll *d* may serve as a light-harvesting pigment in photosynthesis.

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### FLUID SHEAR-STIMULATED DINOFLAGELLATE BIOLUMINESCENCE

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Bioluminescence studies provide insight into the properties of water motion that are stimulatory to flow-sensitive organisms such as dinoflagellates, the most common sources of near-surface oceanic bioluminescence. Previous laboratory studies employing steady flows have characterized the luminescent response of dinoflagellates in terms of shear stress. In the present study, computational and experimental approaches were used to investigate the contributions of shear and acceleration to cells responding in a laminar converging flow field, where regions of high acceleration and shear are spatially separated. Flow-stimulated flashes by the dinoflagellates *Lingulodinium polyedrum* and *Ceratocorys horrida* were used as a near-instantaneous monitor of cell response. By combining video analysis of flash trajectories with computational methods, the location of each stimulated cell was determined and flow parameters at that location were calculated. Based on several criteria, shear stress was considered the flow parameter most stimulatory to cells. For both dinoflagellates species and for all flow rates, essentially all cells responded downstream near the wall where shear stress levels were maximal, and levels of acceleration and extensional stress were as much as two orders of magnitude less than locations away from the wall. Minimum shear stress levels at the cell positions were consistent with response thresholds based on previous studies. Bioluminescence is an excellent tool for examining how organisms respond

to flow at the small temporal and spatial scales relevant to planktonic organisms.

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### NOSTOCACEAN MACRO-MORPHOGENESIS

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A poorly understood feature of nostocacean growth and development is the formation of ordered macroscopic structures from microscopic cells, trichomes, and filaments. Using macro-photography, time-lapse micro-cinematography, light and electron microscopy of *Nostoc* species in pure culture, it has been possible to demonstrate how motility, adhesion and aggregation of photo-induced hormogonia result in macro-morphogenesis of dendroid forms. Red-light induced hormogonia from synchronized cultures aggregate rapidly on agar as tight flowing streams, in patterns responsive to the direction and quality of incident light. Unlike the even textured cell surfaces of heterocystous filaments, the cell walls of swarming hormogonia are covered with a striate mucoid layer containing pili attached to cells of adjacent hormogonia. During differentiation to an aseptate phase, cell wall fusions occur and a gelatinous matrix forms around the enlarging sub-globose cells. Liquid suspensions of hormogonia aggregate in a solid mass following the net centripetal movement of interlaced loops of curved hormogonia attached by adhesive pili. In darkness or dim white light, compressed hormogonial aggregates form erect tree-like (dendroid) macro-structures by photo-tactic reversal of streaming motility. Hormogonia within the aggregates re-organize into streams that push upward into the light, forming structured, positively phototropic protuberances, several millimeters in length. Under weak illumination, the structures become branched with crowns of waving hormogonia. The dendroid morphology is stabilized by deposit of gelatinous material derived from successive cycles of cell-filament development, liberation of heterocysts and formation of dormant cells and trichomes.

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### STEROLS AS BIOMARKERS IN *GYMNODINIUM BREVE*: DISTRIBUTION IN DINOFLAGELLATES

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The sterol composition of marine microalgae has been shown to be a chemotaxonomic property potentially of value in distinguishing members of different

algal classes. For example, members of the class Dinophyceae display sterol compositions ranging from as few as two (cholesterol and dinosterol in *Alexandrium tamarense*) to as many as twelve in certain *Heterocapsa* species. Certain of these are 4-methyl sterols rarely found in other classes of algae. The ability to use sterol biomarkers to distinguish certain dinoflagellates such as the toxic species *Gymnodinium breve*, responsible for red tide events in the Gulf of Mexico, from other species within the same class would be of considerable scientific and economic value. *Gymnodinium breve* has been shown by others to possess two principal novel sterols, (24S)-4a-methylergosta-8(14), 22-dien-3b-ol (ED) and its 27-nor derivative (NED), not previously known to be present in other dinoflagellates. Characterization of free and esterified sterols from laboratory cultures of *G. breve* and of samples from a recent bloom in the Gulf of Mexico has confirmed the predominance of these two sterols. This has prompted a study of the sterol signatures of more than 30 dinoflagellates. ED and NED were shown also to be the primary sterols of the closely related dinoflagellates *G. mikimotoi* and *G. galatheanum*. They are also found as minor components of the more complex sterol profiles of other members of the *Gymnodinium-Peridinium-Prorocentrum* (GPP) taxonomic group. The more widespread distribution of this sterol pattern is consistent with the known close relationship between *G. breve* and *G. mikimotoi*.

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### SPECIES PHYLOGENY OF *COSMARIUM* AND *STAUSTRUM* (DESMIDIACEAE) BASED ON *RBCL* SEQUENCES

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*Cosmarium* and *Staurastrum* are the two most diverse genera of placoderm desmids (Family Desmidiaceae), with approximately 1100 and 800 species, respectively. Phylogenetic analysis of relationships of species has been extremely difficult. In a monograph of North American placoderm desmids, Prescott *et al.* described early phylogenetic work that concluded *Staurastrum* to be polyphyletic and certainly polymorphic. Likewise, *Cosmarium* has also been viewed as polyphyletic, and a number of workers have proposed splitting these genera. The classical view of West and West grouped species within each genus into two divisions and 6–8 sections based on wall features and semicell shape. We sequenced *rbcl* from 18 species of *Cosmar-*

*ium* (2 divisions, 7 sections) and 12 species of *Staurastrum* (2 divisions and 7 sections) and performed a phylogenetic analysis (parsimony, maximum likelihood, bootstrap) using other placoderm desmids and Zygnematales as outgroups. The results exhibit little support for the monophyly of sections or divisions of the two genera. Furthermore, although there is support for the monophyly of clades within each genus, there is also support for a separate clade containing species from both genera.

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#### MEASUREMENT OF MICROALGAL CELL VOLUME BY FLOW CYTOMETRY

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Single cell analysis by flow cytometry is a powerful tool that has been employed to identify many different characteristics of phytoplankton populations. Cell volume is an important physiological component of many cellular processes. We have used a Coulter EPICS XL flow cytometer to measure cell volume in the spheroid dinoflagellate *Amphidinium operculatum* as a function of forward scatter. Cell volume measurements of this alga were quantified as equivalent spherical diameters from a standard curve obtained with latex beads of known diameter. This parameter was used to monitor cell diameter throughout the cell division cycle. In log phase cultures, *A. operculatum* showed increasing cell volumes throughout the light phase and a maximum cell volume concurrent with the onset of cell division late in the light phase. The maximum equivalent spherical diameter measured 14  $\mu\text{m}$ , while the minimum equivalent spherical diameter was 10  $\mu\text{m}$  that occurred late in the dark phase. Stationary phase cultures of *A. operculatum* did not exhibit oscillating cell volumes throughout the diel cycle. Chemical inhibition of the cell cycle using 100  $\mu\text{M}$  olomoucine diminished cell volume changes during the light phase. These results suggest a coupling of size control to the cell division cycle.

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#### IDENTIFICATION OF A CYCLIC AMP-DEPENDENT PROTEIN KINASE IN THE DINOFLAGELLATE AMPHIDINIUM OPERCULATUM

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Cyclic AMP is an important signaling molecule in both prokaryotes and eukaryotes. In eukaryotes, the

action of cyclic AMP is mediated by cyclic AMP-dependent protein kinase (PKA). Efforts to identify PKA in dinoflagellates have met with mixed success in previous studies. In *Amphidinium operculatum*, we have identified the presence of cAMP, and observed cell cycle inhibition by the cAMP pathway inhibitor, isobutyl methylxanthine, suggesting the presence of a eukaryotic cAMP pathway. However, crude lysates of *A. operculatum* failed to show in vitro kinase activity toward kemptide, a specific substrate of mammalian PKA. This may reflect the presence of unidentified low molecular weight inhibitors, because when cell lysates were fractionated by gel filtration chromatography, cAMP dependent kinase activity was identified. The molecular weight of this PKA was 66 kDa, much smaller than bovine PKA (250 kDa), but similar to that reported in the diatom *Cylindrotheca* (78 kDa). Current work is focusing on characterization of subunit structure of the PKA of *A. operculatum*, as compared with bovine PKA.

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#### RELATIVE TOLERANCE OF MAT-FORMING ALGAE TO COPPER AND ITS IMPLICATIONS

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The major method for algae control in the U.S. is the application of copper. Certain groups of phytoplankton, such as the green algae, are known to be more tolerant to copper than groups such as the cyanobacteria. Through experience, commercial applicators suggest that mat-forming algae also differ in their susceptibility to copper, but to this point, data to support these observations have not been available. Laboratory studies in which unialgal cultures of mat-forming algae were exposed to a range of copper concentrations show a clear progression in copper tolerance. The EC<sub>50</sub> values (concentration of copper in mg Cu L<sup>-1</sup> that results in 50% reduction of biomass) were less than 0.003 (*Spirogyra*, *Oedogonium*), approximately 0.050 (*Hydrodictyon*, *Pithophora*, *Rhizoclonium*), and greater than 0.200 (*Oscillatoria*). These data are important to us in the midwest because *Oscillatoria* is becoming a more frequent component of mat-forming algae infestations. The *Oscillatoria* infestations are caused by at least five different species, each of which is very tolerant to copper. Circumstantial evidence suggests that the increasing frequency of this mat-forming cyanobacterium is due to elimination of more sensitive genera (such as *Spirogyra* or *Rhizoclonium*) with heavy copper dosing. In addition to concerns often expressed about copper use, such as buildup in the sediments, toxicity to invertebrates, and possible (although not well verified) resistance of cyanobacteria, another compelling reason to seek al-

ternative approaches to copper is the buildup of copper-tolerant mat-forming algae.

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**PHENOLOGY OF *SCINAIA LATIFRONS* (NEMALIALES; RHODOPHYTA) IN THE SOUTHWESTERN GULF OF CALIFORNIA, MEXICO**

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*Scinaia latifrons* Howe is one of the main species in rhodolith beds between 30 and 90 feet; however, little known about the morphological changes of this species. Consequently, we investigated the phenological changes of the species at San Lorenzo channel from December 1998 to December 1999 (based on bi-weekly sampling during the first 6 months and monthly sampling during the last part of the study). The gametophyte phase of the species is present from December to late May and the sporophyte potentially is present the rest of the year. The peak of the development occurs in April and reproduction occurs in small size plants with two cohorts in the period. This species presents an allometric growth pattern due to the lack of correlation between size and width of the plant or branching patterns. Our data suggest that temperature and light control the development of the plant. Furthermore, we found the spermatogonial structure in the same thallus as the female/carpogonophyte plant indicating that this species is monoecious (contrary to what was considered previously). This suggests that some taxonomic problems are present in this species in relation with *S. cottoni*.

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**PHYLOGENETIC AND TAXONOMIC REASSESSMENT OF THE DELESSERIACEAE (CERAMIALES, RHODOPHYTA) BASED ON TWO MOLECULAR DATA SETS**

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The current system of classification of the Delesseriaceae rests largely on Kylin's treatment which encompass the subfamilies Delesserioideae with five 'Groups' and Nitophylloideae with six 'Groups'. In this study we test the Kylin system based on phylogenetic analyses inferred from two molecular data sets and morphological evidence. A total of 145 delesseriacean taxa worldwide were sequenced for chloroplast-encoded *rbcL*, and 82 taxa for the nuclear-encoded large subunit ribosomal RNA gene (LSU). Three large clades are identified in the combined data sets,

one of which corresponds to the Delesserioideae, one to a narrowly circumscribed Nitophylloideae, and the third to a new subfamily comprising the remainder of the Nitophylloideae sensu Kylin. Belonging to the Delesserioideae are an expanded *Hemineura* group that includes *Hemineura*, *Patulophycus*, *Marionella*, *Laingia*, *Botryocarpa* and possibly *Pseudophycodrys*, a *Caloglossa* group, a *Hypoglossum* group that includes *Hypoglossum*, *Branchioglossum*, *Bartoniella*, *Zellera*, *Phytomorphora*, and *Chauwiniella*, a *Delesseria* group with *Delesseria*, *Membranoptera* and possibly *Grinnellia* (but not *Apoglossum*) and most southern hemisphere species of *Delesseria* which are properly placed in *Paraglossum* J. Agardh 1898. The revised Nitophylloideae contains *Nitophyllum*, *Valeriemaya*, *Polyneuroopsis*, *Calonitophyllum* and also *Martensia* and *Opephyllum*. The unnamed subfamily includes the *Phycodrys* group with *Phycodrys* and *Polyneura*, the *Cryptopleura* group with species placed in *Cryptopleura*, *Hymenena*, *Acrosorium* and *Botryoglossum*, the Myriogrammeae with *Myriogramme* and *Haraldiophyllum*, and the Schizoseriideae with *Schizoseris*, *Neuroglossum*, *Drachiella*, *Abroteia*, and South American species of *Platyclinia*. This research promotes the correlation of molecular and morphological phylogenies with biogeographic hypotheses for the family.

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**PHYLOGENY OF THE EUGLENOPHYTES: ANALYSIS USING SSU rDNA AND ULTRASTRUCTURAL CHARACTERS**

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The use of both molecular and morphological data to determine relationships among the euglenoids is vital for a complete understanding of their phylogeny, and the development of an accurate taxonomy. Analyses of the SSU (18S) rDNA from 12 euglenoid genera have resulted in tree topologies that are in agreement with many defining morphological characters. The euglenoid lineage is formed by phagotrophic euglenoids at its base, followed by the divergence of phototrophs that in-turn gave rise to osmotrophs. The photosynthetic lineage is anchored by euglenoids with two emergent flagella, *Eutreptia* and *Eutreptiella*, while the remainder of the lineage is composed of euglenoids with a single emergent flagellum. Among the photosynthetic euglenoids with a single emergent flagellum those that secrete mucilaginous stalks, *Colacium*, or form a lorica, *Trachelomonas* and *Strombomonas*, are closely associated. The remaining photosynthetic genera *Euglena*, *Phacus*, and *Lepocinclis* are intermixed with each other and the osmotrophic genera *Astasia*, and *Khawkinea*. Hence, they are not monophyletic, sensu Hennig. To reinforce molecular phylogenies, a robust morphological character database is necessary. For taxa with complex internal structures complete

serial reconstruction is required. Serial reconstruction of the flagellar and feeding apparatuses in *Ploecia costata* illustrate this necessity. Originally described as having both an MTR (Type I) and a Type II feeding apparatus, reconstruction has shown *P. costata* to have a single, Type II, feeding apparatus. Moreover, the Type II now appears to be an autapomorphy for *Ploecia* species, while euglenoid feeding apparatuses, in toto, appear to form a continuum of structural types.

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**PHENOLOGY OF PORPHYRA PENDULA (BANGIALES; RHODOPHYTA) IN THE SOUTHWESTERN GULF OF CALIFORNIA, MEXICO**

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*Porphyra pendula* Dawson is an endemic species of the mid-intertidal zone in the Gulf of California. Based on surveys from the Phycological Herbarium of UABCS since 1994, we found an extensive population of this species in Punta Coyote, south of La Paz. Here, we assess the phenology of the gametophyte phase from December of 1998 to June of 1999 with visits every 2 weeks. Our results show significant temporal and spatial changes in length and width of the plant, with some quadrants displaying two peaks in development (January and March) and other quadrants with a peak only in January. A significant correlation existed between length and width of the blade and is linked with plant maturation, observations which strongly support the isometric growth rate of the species. No differences between exposed and protected areas were observed suggesting stronger differences between quadrants. Differences plants in length and in color existed between male and female plants; the males were smaller and yellow and females/carposporophyte were larger and red-purple. Most of the thalli were reproducing during the study, with a greater proportion of females than males (ca. 2:1). Our results suggest that the morphological changes in this species may be related with temperature and day length.

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**GRAZER-INDUCED DEFENSE IN SCENEDESMUS OBLIQUUS (CHLOROPHYCEAE)**

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The non-spiny *Scenedesmus obliquus* may express considerable phenotypic plasticity in response to changes

in their environment. When exposed to chemicals released from the grazer *Ceriodaphnia*, unicellular *S. obliquus* populations were rapidly transformed into colonial ones. The morphological response appeared strongly correlated with the amount of algae grazed upon by the *Ceriodaphnia*. The induced *S. obliquus* colonies were protected against grazing, as were large spined *Scenedesmus*, whereas unicellular *S. obliquus* and spined *Scenedesmus* remained vulnerable to grazing by *Ceriodaphnia*. Growth rates of unicellular and induced-colonial *S. obliquus* populations were identical. However, sinking rates were significantly higher for the induced-colonial *S. obliquus*. Hence, the phenomenon of grazer-induced colony formation in *S. obliquus* can be interpreted as an inducible defense at the expense of higher sinking losses.

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**THE TYPE SPECIES OF CERAMIUM (RHODOPHYTA), CERAMIUM VIRGATUM ROTH: TYPIFICATION AND PHYLOGENY**

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The type species of *Ceramium* is *Ceramium virgatum* Roth (1797, Cat. Bot I: 148, pl. VIII, fig. 1). This has been treated as a probable synonym of *Ceramium nodulosum* (Lightfoot) Ducluzeau, one of the species formerly confused under the illegitimate name *Ceramium rubrum* (Hudson) C. Agardh. However, in 1996 Silva showed that *C. nodulosum* (Lightfoot) Ducluzeau is a later homonym of *C. nodulosum* de Candolle 1805, and advocated conserving *C. rubrum* with a neotype. As an alternative to this, we investigated whether the name *C. virgatum* might be available for this species. The lectotype of *C. virgatum* is Roth's illustration of a much-branched, fully corticate, *Ceramium* specimen from Eckwarden, North Sea, which could represent any member of the '*C. rubrum*' group. We obtained material resembling Roth's, from the North Sea island of Helgoland, as a potential epitype. Its *rbcl* sequence was aligned with sequences of all *C. rubrum*-like species in the British Isles, i.e. *C. secundatum*, *C. botryocarpum*, *C. pallidum* and '*C. nodulosum*'. The Helgoland material was clearly conspecific with '*C. nodulosum*' from Ireland (0.08% divergence). We therefore suggest that the name *C. virgatum* Roth should be employed for this species. Phylogenetic analyses of these sequences aligned with various representatives of the tribe Ceramieae, placed the North Atlantic *C. rubrum*-like species in two well-supported clades. In one of these, *C. virgatum* is basal to *C. secundatum* and *C. botryocarpum*. The other clade consists of *C. pallidum* and a partly ecorticate species resembling *C. diaphanum*.

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### INTER-FAMILY USE OF MICROSATELLITE MARKERS IN LAMINARIALES: THE EXPERIENCE BETWEEN *LAMINARIA DIGITATA* AND *LESSONIA* SPP.

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Among the co-dominant molecular markers, microsatellite loci have a number of advantages in population genetic studies. However, the different methods to hunt these markers are expensive, time consuming, and they require sophisticated laboratory equipment. Using in one species the microsatellites primers originally described for another one saves time and reduces costs. Examples in the literature revealed that microsatellites described for *Gracilaria gracilis* from France have not worked for other members of the Gracilariales. The results were not very promising, at least for *Gracilaria chilensis* from Chile. In this study, a number of microsatellite loci described for *Laminaria digitata* (Laminariaceae) from France were amplified and sequenced in two *Lessonia* (Lessoniaceae) species from Chile. Preliminary results show a partial conservation of both, flanking and tandem repeat regions. Some polymorphism has also been detected in *Lessonia* spp. The higher molecular affinity (conservation of primer sites) observed in species belonging to different families of brown algae, respect to that observed among species of the same genus in the red algae, is surprising. Such a result is strikingly counter-intuitive when observing the morphological disparity among the Laminariales, respect to the similarity observed in the Gracilariales. It also reminds one of an earlier discussion by Gary Saunders about "When a family is not a family".

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### PLASTID FATTY ACID BIOSYNTHESIS IN THE DIATOMS *NITZSCHIA ALBA* AND *NITZSCHIA LAEVIS*

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The role of the plastid in fatty acid biosynthesis in the non-photosynthetic diatom *Nitzschia alba* was studied and compared to that in the photosynthetic, closely related *Nitzschia laevis*. Transmission electron microscopy was used to analyze structural features of the plastid that may be relevant to biochemical function. Typical of a photosynthetic diatom, *N. laevis* had a chloroplast envelope composed of four membranes,

and had abundant chloroplast ribosomes. The leucoplast of *N. alba* also had a multi-membrane envelope, chloroplast ribosomes, and a genome that encodes plastid specific proteins. This suggested that the plastid of *N. alba* may still possess the biochemical functions of the chloroplast, aside from photosynthesis. To determine whether plastidial fatty acid biosynthesis occurred in *N. alba*, the response of the two diatoms to the compound thiolactomycin was compared. Thiolactomycin has been shown to effect keto-acyl-ACP-synthases, and specifically inhibits the plastidial fatty acid biosynthetic pathway. While growth of *N. alba* was not impacted by thiolactomycin as in *N. laevis*, neutral lipid accumulation and fatty acid composition was impacted by thiolactomycin in both organisms. These findings suggest that the plastidial fatty acid biosynthetic pathway does exist in the leucoplast of *N. alba*, although it lacks photosynthetic capacity.

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### SYSTEMATICS AND PHYLOGEOGRAPHY OF THE INVASIVE RED ALGA, *POLYSIPHONIA HARVEYI*

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*Polysiphonia harveyi* Bailey was first described from Connecticut in 1848 and morphologically similar species, such as *P. japonica* and *P. strictissima*, have subsequently been described many times from different localities such as Japan and New Zealand, placing the taxonomy and nomenclature of this morphologically variable species in confusion. *Polysiphonia harveyi* is regarded as an alien in the British Isles and the northeastern North Atlantic and is reported to be spreading rapidly. The first confirmed collection of *P. harveyi* from the British Isles was in 1908; the source of the British introduction remains unknown. In conjunction with breeding data, *rbcL* sequences for samples of *P. harveyi*, *P. strictissima*, *P. japonica* and *P. akkeshiensis* collected from New Zealand, Atlantic and Pacific North America, Japan and Europe, reveal that *P. harveyi*, *P. japonica* and *P. akkeshiensis* constitute a single biological species. The correct name for this is *P. harveyi*, whilst *P. strictissima* from New Zealand is a sibling species, distinct from *P. harveyi*. The center of genetic diversity of *P. harveyi* is in Japan. The original source of the introduced *P. harveyi* in the British Isles, Europe and Nova Scotia appears to be Hokkaido, Japan. Separate introductions from a single source population into New Zealand and Pacific and Atlantic North America from Honshu, Japan, apparently have occurred.

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**THE TYCHOPELAGIC DIATOM, *PARALIA SULCATA*, AS PALEOINDICATOR SPECIES IN COASTAL MARINE ENVIRONMENTS**

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*Paralia sulcata* is a diatom commonly found in both the plankton and benthos of coastal environments. This species is heavily silicified and, thus preserves well in sedimentary records making it a potentially useful paleoindicator species. However, its tychope-lagic nature and its association with a wide range of environmental conditions have made detailed paleo-ecological interpretations complicated. High-resolu-tion sediment records from coastal fjords in both Can-ada and Sweden show variations in the abundance and morphology of *P. sulcata* that provide evidence of changes in benthic habitat distribution and surface water properties in the fjords on timescales of decades to centuries. These studies suggest that *P. sulcata* can be an important paleoindicator species when inter-pretations are made in the context of its complex ecology.

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**PHYLOGENY OF THE GENUS *CHLOROCOCCUM*: A STUDY OF 18S AND 26S rRNA GENE SEQUENCES**

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Phylogenetic studies of 18S rRNA gene sequence data support an alliance of the green algal genus, *Chlorococ-cum*, with other chlamydomonadalean taxa that pro-duce walled, motile stages. Moreover, these phyloge-netic studies indicate that *Chlorococcum* is not monophyletic, but rather falls into two distinct lin-eages. However, these molecular phylogenetic studies were based on only a few taxa in a genus that exhibits marked habitat diversity. In particular, numerous spe-cies of *Chlorococcum* currently in the UTEX collection have been isolated from naturally acidic environ-ments. Except for a few groups (e.g., desmids), acidic environments are not generally regarded as niches for widespread exploitation by green algae. A new investi-gation of diversity among species of *Chlorococcum* in-cluded broader taxon and data sampling schemes. Se-quence data from both the nuclear-encoded 18S and 26S rRNA genes were collected from 17 different UTEX and SAG isolates of *Chlorococcum*. In addition, controlled growth experiments at varying acidity (pH 5.0, 6.0, and 8.0) were conducted. Results of these in-

vestigations suggest that the *Chlorococcum*-like mor-phology has evolved in at least three lineages. Further-more, these data suggest that the ability to grow opti-mally at low pH has independently evolved in sev-eral lineages.

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**COMPARING RESPIRATORY (ETS) ACTIV-ITY OF BACTERIA ATTACHED TO PHY-TOPLANKTON AND FREELY SUSPENDED IN SURROUNDING SEAWATER**

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We conducted investigated respiratory (ETS) activity of bacteria attached to phytoplankton and living free in surrounding seawater collected from coastal waters at Charleston, Oregon, USA. We hypothesized that the surface of a phytoplankton cell provides a habitat that permits enhanced metabolic activity in attached bacteria. In order to test this hypothesis, the number of bacteria that reduce 5-Cyano-2,3-di-tolyl tetrazo-lium chloride (CTC) were compared to the total number of bacteria, assessed by the nucleic acid stain, 4',6-diamidino-2-phenylindole (DAPI). Two-hour incu-bations with the CTC dye and subsequent staining al-lowed for direct viewing of the position and the num-ber of ETS active versus inactive bacteria in each sample. We found that the percentage of active bacte-ria attached to the phytoplankton cells' surface (42%) was 10 times that of bacteria freely suspended in the surrounding seawater (4%). Previous investigations of ETS activity of freely suspended bacteria found that 1% to 8% are actively respiring while non-living parti-cle associated bacteria showed that 20 to 35% of bac-teria are actively respiring. Our results suggest that bacteria in coastal waters are substrate limited, and the extracellular products of photosynthesis may be more important than is generally realized. The sur-faces of phytoplankton cell surfaces may provide habi-tat and substrates for a disproportionate share of bac-terial production in coastal waters.

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**SEASONAL GROWTH AND NUTRIENT ECO-PHYSIOLOGY OF *ECKLONIA RADIATA* FROM DOUBTFUL SOUND, FIORDLAND, NEW ZEALAND**Miller, S. M.<sup>1</sup>, Hurd, C. L.<sup>1</sup> & Wing, S.<sup>2</sup><sup>1</sup>*Department of Botany, University of Otago, Dunedin, New Zealand;* <sup>2</sup>*Department of Marine Science, Univer-sity of Otago, Dunedin, New Zealand*

The kelp, *Ecklonia radiata*, dominates the sublittoral zone within the Doubtful Sound complex. Doubtful Sound is one of a series of fourteen fiords indenting

the southwestern coast of the South Island, New Zealand. A feature of these fiords is the freshwater layer that resides atop the salt water, creating a unique marine environment. Mature sporophytes were collected from three sites (two outer sites towards the coastline and one site within the fiord) over a fourteen-month period (October 1998 to December 1999). Growth rates, measured using the hole punch technique, were greatest in spring at all three sites. The nutrient eco-physiology of mature *E. radiata* was investigated by measuring inorganic nitrogen ( $\text{NO}^{-3}$  and  $\text{NH}^{+4}$ ) uptake rates at eight concentrations (2–64  $\mu\text{M}$ ). Soluble tissue nitrogen ( $\text{NO}^{-3}$  and  $\text{NH}^{+4}$ ) and tissue carbon:nitrogen (C:N) ratios were also determined. At all sites, *E. radiata* showed higher uptake rates for ammonium than for nitrate. In winter, soluble tissue nitrogen was lower at the two outer sites compared to the inner site, while in summer, levels at all sites were similar but had decreased from the winter values. C:N ratios greater than 20 were observed at all sites, increasing in summer to a peak in autumn, suggesting *E. radiata* may be nitrogen limited, particularly in late summer. Environmental parameters (photon flux density, water temperature, rainfall and wind) were measured, as was sea water velocity around the primary blade of *E. radiata*, and multivariate correlations used to determine which (if any) environmental parameter exerted greatest influence on eco-physiological parameters accessed.

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#### THE EVOLUTION OF THERMOTOLERANCE IN HOT SPRING CYANOBACTERIA OF THE GENUS *SYNECHOCOCCUS*

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The extension of ecological tolerance limits may be an important mechanism by which microorganisms adapt to novel environments, but it may come at the evolutionary cost of reduced performance under ancestral conditions. We combined a comparative physiological approach with phylogenetic analyses to study the evolution of thermotolerance in hot spring cyanobacteria of the genus *Synechococcus*. Among the twenty laboratory clones of *Synechococcus* isolated from collections made along an Oregon hot spring thermal gradient, four different 16S ribosomal RNA gene sequences were identified. Phylogenies constructed using these sequence data indicated that the clones were polyphyletic but also that three of the four sequence groups formed a clade. Differences in thermotolerance were observed for clones with different 16S rRNA gene sequences, and comparison of these physiological differences within a phylogenetic frame-

work provided evidence that more thermotolerant lineages of *Synechococcus* evolved from less thermotolerant ancestors. The extension of the thermal limit in these bacteria was correlated with a reduction in thermal niche breadth, which may have implications for the geographic distributions of these organisms. This study illustrates the utility of using phylogenetic comparative methods to investigate how evolutionary processes have shaped historical patterns of ecological diversification in microorganisms.

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#### RELATING PHYTOPLANKTON BIOMASS AND PRODUCTION TO EPISODIC PHYSICAL FORCING IN SOUTHEASTERN LAKE MICHIGAN

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Spatial and temporal dynamics in phytoplankton reflect of the combined effects of the physical and chemical environments and associated biological responses. Although alterations in phytoplankton are well-documented for a variety of lentic waters, the exact linkages between environmental forcing and phytoplankton assemblages remain poorly understood (particularly for coastal systems). A recurrent sediment resuspension event occurs every late winter/early spring in southeastern Lake Michigan, often extending greater than ten km in width and 300 km in length. Inherently, such a large-scale and dramatic physical process would be thought to dramatically influence phytoplankton assemblages; however, linkages between the turbidity plume and phytoplankton assemblages have been postulated, but never verified. As such, the episodic nature of the plume provided an opportunity to examine the effects of a short-term physical forcing event on coastal phytoplankton in relation to more persistent, seasonal meteorological forcing. Lake phytoplankton assemblages within and outside of the RCP were examined during the spring isothermal period from 1998 to 2000. Here, we describe results from the 1998 and 1999 field seasons characterizing the distribution of phytoplankton biomass and composition within and adjacent to the RCP and their relationship to particulate and dissolved constituents. In addition, the spatial and temporal patterns in production and photosynthetic characteristics of the phytoplankton community are examined.



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**ALGAL BIOREMEDIATION OF THE BERKELEY PIT LAKE**

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Ongoing research is unraveling the intricacies of the microbial ecology of the Berkeley Pit Lake System, with ever increasing information becoming available regarding the diversity of Algae, Protists, Fungi and Bacteria that inhabit this mine waste site. Defining the baseline community structure has been the first step not only toward understanding the interactions of the different groups of organisms, but also toward assessing any improvement in biodiversity within the biotic community. Now that this first step has begun, some of these extremophiles, specifically algae, that have been isolated from the Berkeley Pit Lake System are being used as a potential solution for bioremediation. The specific objectives of this research are fivefold: 1) To evaluate the bioremediative potential of our four most rapidly growing species: (*Chromulina freiburgensis* Dofl., *Chlorella ellipsoidea* Gerneck, *Chlorella vulgaris* Beyerinck and *Chlamydomonas acidophilla* Negoro) in Berkeley Pit Lake System Water with the additions of  $\text{NaNO}_3$  and  $\text{NaPO}_4$  by using an experimental matrix. This matrix will be used to estimate the minimum nutrient concentrations that would be necessary to achieve the maximum growth of algae and maximum bioremediation of the Berkeley Pit Lake System. 2) To determine which combination of nutrients will stimulate growth of the best bioremediator of our four isolated species in natural Berkeley Pit Lake System waters. In other words, what nutrient combination will give the best bioremediator a competitive edge over the other species. If time permits, different species may be grown in combination to determine if there are synergistic effects (protocooperation) between/among species. 3) To determine a temperature profile for these four species in order to determine their optimal growth temperature in Berkeley Pit Lake System water. 4) To continue to isolate organisms from the Berkeley Pit Lake System and determine their bioremediative potential. 5) Monitor algal and bacterial counts from a profile of Pit Lake System waters. The results to date will be presented for this conference.

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**DIVERSITY AMONG TETRASPORALEAN TAXA: EVIDENCE FROM ULTRASTRUCTURE AND MOLECULES**

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Recent phylogenetic studies of tetrasporalean exemplars using 18S rDNA revealed the extent of diversity among taxa in this non-monophyletic group (Booton et al. 1998). In particular, two distinct tetrasporalean lineages were identified; one group allied with chlamydomonadalean taxa and the other group comprising taxa now placed in a separate order, the Chaetopeltidales. Using these observations as a framework, a new investigation of diversity among a broader sampling of putative tetrasporalean genera (e.g. *Asterococcus*, *Chloranomala*, *Chlorophysemma*, *Gloeocystis*, *Gloeodendron*, *Palmella*, *Paulschulzia*, *Physocytium*, *Schizochlamys* and *Tetraspora*) was undertaken. Phylogenetic studies of both 18S and 26S rDNA were compared with ultrastructural investigations of vegetative cells. Molecular phylogenetic analyses corroborate the earlier 18S rDNA results, but also reveal additional diversity. The new data raise doubts regarding the monophyly of two genera, *Palmella* and *Tetraspora*. The new data also link two enigmatic green algal genera, *Physocytium* and *Heterochlamydomonas*, in a long-branch lineage within the Chlamydomonadales. Another enigmatic genus, *Schizochlamys*, is allied with *Bracteacoccus* in the Sphaeropleales. Lastly, *Chloranomala* is resolved as an ally of *Paulschulzia*, *Tetraspora* sp., and the green flagellate, *Lobomonas*. Comparison of pyrenoid ultrastructure generally supports the molecular phylogenetic analyses, suggesting that this non-molecular character will be a useful marker for broad phylogenetic studies of chlamydomonadalean taxa. (Supported by NSF grant, DEB 9726588)

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**CHEMICALLY INDUCED ECDYSIS AND ISOLATION OF NUCLEI FROM THE DINOFLAGELLATES GONYAULAX POLYEDRA AND GONYAULAX TAMERENSIS**

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Armored dinoflagellates such as *Gonyaulax* sp. are not good candidates for isolation of intact clean nuclei, due to difficulty in breaking the cells. Previously, isolation of nuclei from *Gonyaulax* was achieved using vigorous cell disruption by ultrasonication (Rizzo & Hastings, unpublished observations). A detergent method for production of spheroplasts from *Gonyaulax* was published some time ago (Adamich & Sweeney, 1976), but was not reproducible (Rizzo & Hastings, Morris & Rizzo, unpublished observations). We describe here a modification of the method described by Adamich & Sweeney that is highly reproducible. Ecdysis and production of intact dinoflagellate spheroplasts with a yield of virtually 100% within 5 minutes, has been achieved through the use of anionic and nonionic detergents in hypotonic buffers. This method of ecdysis followed by a gentle sphero-

plast breakage in a hypotonic buffer allowed the release of nuclei for later biochemical analysis with minimal damage or breakage. The nuclei are subsequently purified by centrifugation in a Ficoll discontinuous gradient.

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### STRUCTURAL AND SEQUENCE SIGNATURES OF NUCLEAR SSU rRNA DEFINE TAXONOMIC LEVELS WITHIN THE RHODOPHYTA

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Over 400 nuclear SSU rRNA sequences representing all orders of the Rhodophyta were aligned and analyzed using comparative sequence analysis. Numerous nucleotide positions and structural elements were found that delineated various taxonomic groups. The 1245 region (*E. coli* numbering) contained a loop that differed in size between two conserved helices and clearly separated the Florideophyceae [3 nt (>95% of 268 sequences)], Bangiales [13 to 14 nt (100% of 116 sequences)] and remaining Bangiophyceae including the Cryptophyta nucleomorphs [four to eight nt (100% of 32 sequences)]. In addition, members of the Thoreaceae were found to have additional helices in the 650 and 1139 region of which a corresponding structure was not present in any other red algal SSU rRNA gene sequence. Base-pair and nucleotide signatures differentiated the Bangiales, Florideophyceae, Bangiophyceae (not including Bangiales) and Hildenbrandiales at three levels of comparison: within the Rhodophyta (>400 sequences), the Eukaryota (not including Rhodophyta; >1300 sequences) and three kingdom (Archaea, Bacteria, 2 organelles, Eukaryota; >7000 sequences). For example, all members of the Hildenbrandiales have a change in the base-pair 512:539 that is a region of functional importance. Sequences from the Eukaryota, Archaea, Bacteria and two organelles have a C:G or a U:A in this position whereas the Hildenbrandiales have a C:A pair. This analysis raises the possibility of utilizing structural features of nuclear SSU rRNA and sequence signatures to support and delineate phylogenetic clades within the Rhodophyta.

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### COMBINED EFFECTS OF MACRONUTRIENTS AND METALS ON THE ADRIATIC GREEN ALGA *ULVA RIGIDA* C.AG.

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The ephemeral green alga *Ulva rigida* C. Ag. is common in polluted and eutrophicated sites of the northern Adriatic. Its tolerance and responses to the combined effects of excess macronutrients (nitrates, phosphates) and metals (Mn, Co, Zn) that are essential micronutrients were studied *in vitro* regarding growth expressions, survival, elemental composition (N, C, P), and metal accumulation. Plants belonging to vernal/estival and autumnal populations were compared. Excess nutrients were applied together with the single metals. Nitrate and phosphate were used separately and in a dual combination. In control samples that lacked metals, it was obvious that plants belonging to vernal and estival populations grew better in phosphate than in nitrate enriched media and that the opposite was true for autumnal plants. The presence of metals altered the responses of *U. rigida* to excess nutrients. In some of the samples a relationship between Mn accumulation and growth was indicated, Mn promoting growth. Metal accumulation was greater in the autumnal than in the vernal and estival plants, being the highest for Mn and lowest for Zn. A comparison between experimental series of different duration revealed that the metal accumulation increases time-dependently for Mn and Co. The N and P content of the plants followed the nutrient enrichment of the media. Autumnal plants accumulated more N and P than vernal and estival ones. It was obvious that the presence of metals influences the nitrogen uptake of *U. rigida*, in the sequence Mn-Co-Zn. The presence of Mn promotes nitrogen uptake and that of Zn is inhibitory.

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### SEASONAL SUCCESSION OF PHYTOPLANKTON IN LAKE PRINCE, SUFFOLK, VIRGINIA: PRELIMINARY REPORT

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Lake Prince is a reservoir lake that provides the region with drinking water, recreational boating, and fishing. The Virginia Department of Game and Inland Fisheries has an interest in the quantity and quality of phytoplankton production in the lake, especially regarding the health of fish populations. Another concern in this reservoir is oxygen availability, and aerators have been installed in the main body of the lake. A year-long examination of the phytoplankton community structure is being conducted. Duplicate surface water samples are collected monthly from

three stations, along with physical and chemical baseline data. Community structure and dominance are being examined with regard to the physical and chemical parameters, as well as seasonal climate changes. Cyanobacteria and Cryptophytes are the dominant spring flora. Diatoms and Chrysophytes are sub-dominant populations.

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### PRELIMINARY STUDIES OF SEASONALITY, ECOLOGY, AND SPECIES COMPOSITION OF ULVOID ALGAL BLOOMS IN WASHINGTON STATE

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Blooms of green macroalgae can devastate important finfish and shellfish habitats. *Ulvaria obscura*, a relatively unstudied green alga, is a major contributor to these blooms in the San Juan Islands, Washington State, USA. The biomass and productivity of this and other ulvoid algae were measured seasonally for two years. Experiments comparing the growth rates, responses to desiccation, photoacclimation, and grazer preference of *U. obscura* and *Ulva fenestrata* were conducted. *Ulvaria* blooms tended to occur in the subtidal while *Ulva* blooms were often intertidal. Both genera bloomed between late June and September. Despite their superficial similarity, *Ulvaria* and *Ulva* display markedly different physiological and ecological responses. *Ulva* was capable of faster growth, had higher rates of photosynthesis, and was more desiccation tolerant than *Ulvaria*. *Ulvaria*, however, appears to be more resistant to grazing than *Ulva*.

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### CONFUSING CONVERGENT MORPHOLOGIES: DIVERSITY AND DIFFICULTIES IN NEW ZEALAND ERYTHROPELTIDALES AND BANGIALES

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An examination of New Zealand species of *Porphyra* and *Bangia* has led to the discovery of unexpectedly high diversity. In excess of 30 species of *Porphyra* and 5 species of *Bangia* can be distinguished, based on 18S rDNA sequence data. It is possible to develop a workable taxonomic framework to distinguish species of *Porphyra* found within this geographic region using a range of characters (morphological, anatomical, biochemical, physiological, life history characteristics). However, the taxonomic distinctions do not necessarily

reflect phylogenetic relationships revealed by sequence analyses: convergence in character states is obscuring relationships. In the course of our work we have discovered that four species, originally described on the basis of traditional morphological and anatomical characters as *Porphyra* or *Bangia*, are more closely related to members of the order Erythropeltidales than to the Bangiales. These results are challenging us to consider new ways of describing and defining these organisms. We are finding convergence in character states, not only within *Porphyra* sens. lat. But spanning both the Bangiales and Erythropeltidales, which confounds both taxonomic distinctions and also phylogenetic hypotheses based on morphology and anatomy. This confusion could be countered effectively by the inclusion of molecular sequence data based on vouchered or type material in descriptions of taxa in the orders Bangiales and Erythropeltidales. This would allow conclusive identification of collected material by appropriate molecular tests, which are quicker and more direct identification tools than, for example, using characters drawn from the growth of these organisms under controlled culture conditions.

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### THE PHYLOGENY OF CAULERPA BASED ON rDNA INTERNAL TRANSCRIBED SPACER SEQUENCES

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Phylogenetic hypotheses for the pantropical marine green algal genus, *Caulerpa*, were inferred based on analyses of nuclear-encoded rDNA internal transcribed spacer (ITS) sequences. Results of these analyses were used to assess the correspondence between rDNA phylogeny and traditional sectional taxonomy, to identify synapomorphic morphological characters (including assimilator morphology and chloroplast ultrastructure), and to examine marine biogeographic hypotheses for the genus. Ribosomal DNA ITS sequences were aligned for thirty-three species and intraspecific taxa of *Caulerpa*. Results indicate limited correspondence between phylogeny and sectional taxonomy for the genus, (e.g., the sections Filicoideae and Sedoideae were not monophyletic). In contrast, chloroplast morphology could be mapped to the tree topology with limited homoplasy. Pantropical isolates of the filicoidean species, *Caulerpa sertularioides* and *Caulerpa mexicana* each formed monophyletic groups. *Caulerpa reyesii* was included as a derived taxon within the *Caulerpa taxifolia* clade, suggesting that these species were conspecific and affirmed the lack of correspondence between phylogeny and assimilator morphology. Isolates and various intraspecific taxa of *Caulerpa racemosa* did not form a monophyletic group. Instead, these taxa formed a heterogeneous assemblage with other sedoidean and

flicoid taxa. Within the *C. sertularioides* clade, Caribbean and Atlantic isolates formed a basal paraphyletic group, whereas eastern and western Pacific isolates formed a more derived monophyletic group. Therefore, these results are not consistent with an Indo-West Pacific origin of this species.

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#### FINE STRUCTURE OF *PHACUS SPLENDENS* POCHMAN (EUGLENOPHYTA)

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The general fine structure of *P. splendens* Pochman (Section Pleurapsis) is described. This Section comprises a series of species with very distinctive features unshared by the majority of the members of the genus. The cells are pear-shaped in frontal view, rounded at the anterior end, narrowed posteriorly tapering in a long twisted tail. In lateral view, the anterior margins are asymmetric, undulate to serrate. The pellicle is strongly striated, with ribs helically arranged, oriented to the left, concavely depressed between adjacent ribs. In transverse section, the pellicle outline shows a single microtubule in the middle of each rib, on the base of a central sulcus. The other two are located one at each side of the sulcus. Among other distinguishing characteristics, the most remarkable feature is the presence of a single chloroplast, a fact never reported in this genus before since the minimal observed number is two and most of the *Phacus* species have been described as having numerous, small and discoid chloroplasts. In longitudinal section, a large, leaf-like parietal chloroplast is observed. It extends along the entire cell, bearing three prominent cup-shaped paramylon caps on the external face. In transverse section is C-shaped. The unusual shape and localization of the chloroplast and paramylon in this species resemble descriptions of *Cryptoglena pigra*, suggesting a closer relationship to this organism than to other members of *Phacus*.

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#### PHYLOGENY OF THE BANGIOPHYCIDAE (RHODOPHYTA) AND THE SECONDARY ENDOSYMBIOTIC ORIGIN OF ALGAL PLASTIDS

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The Rhodophyta are composed of the subclasses Bangiophycidae and Florideophycidae. Two evolutionarily interesting features of the Bangiophycidae are: (1) they are the ancestral pool from which the more morphologically complex taxa in the Florideophycidae have arisen and (2) they are the sources of the plastids, through secondary endosymbioses, for the Cryptophyta, Haptophyta, and the Heterokonta. To understand Bangiophycidae phylogeny and to gain further insights into red algal secondary endosymbioses, we sequenced the plastid-encoded small subunit ribosomal DNA coding region (ssu rDNA) from nine members of this subclass and from two members of the Florideophycidae. Phylogenetic analyses were performed with other red algal plus chlorophyll *a+c* algal plastid ssu rDNA coding regions. Our results are consistent with a monophyletic origin of the Florideophycidae forming a sister group of the Bangiales. The Bangiophycidae is of a paraphyletic origin, and orders such as the Porphyridiales polyphyletic and distributed over three independent red algal lineages. The plastids of the heterokonts are most closely related to members of the *Cyanidium* - *Galdieria* group of Porphyridiales and are not directly related to cryptophyte and haptophyte plastids. The phylogenies provide strong evidence for the independent origins of these "complex" algal plastids from different members of the Bangiophycidae.

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#### SEPARATING POPULATION STRUCTURE FROM POPULATION HISTORY IN *ASCOPHYLLUM NODOSUM* (FUCALES)

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*Ascophyllum nodosum* is dominant seaweed along many rocky intertidal shores throughout the North Atlantic. Next to the kelps, fucal taxa such as *Ascophyllum* are the largest macrophytes and provide important habitat for invertebrates. Understanding the underlying genetic structure of natural populations over a range of spatial scales can reveal how the causes of structure may change with scales. Separating population history from population structure may also be elucidated. The analysis is based on six polymorphic microsatellite loci and >1000 individuals. Strong genetic structure at small spatial scale was found and is consistent with demographic models based on long-lived individuals, low recruitment and many sib matings. At large spatial scales only weak population differentiation was found. This is consistent with recent

recolonization of the North Atlantic following the last glacial maximum.

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**PICOPHYTOPLANKTON SEASONAL CYCLE AT THE SIO PIER, LA JOLLA, CALIFORNIA**

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The abundance of phycoerythrin-containing cyanobacteria and picoeukaryotes in water samples from the Scripps Institution of Oceanography pier have been followed at least weekly for more than two years using flow cytometry. These cyanobacteria show a seasonal cycle with generally lower cell numbers during the winter, a "bloom" as water temperatures increase, and higher cell numbers during the summer. However the population abundance appears to be more variable and the magnitude of the annual change in cell number is less than reported for coastal Massachusetts by Waterbury et al. (1986). Isolates have been obtained from pier samples and genetic characterization using rpoC1 (RNA polymerase) sequence data is in progress. The PUB:PEB chromophore ratios of isolates assayed using fluorescence excitation spectra range from about 0.4 (low PUB) to 0.7 (mid-PUB) for isolates grown under white light. The physiological and genetic characterization of isolates is being used to examine the similarities and differences of cyanobacterial populations from different coastal regimes. Similarly a picoeukaryote has been isolated that has a flow cytometric signature similar to the natural population. It appears to be a small nonmotile prasinophyte.

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**INTERACTION OF CHLOROPLAST AND VACUOLES IN *CHLAMYDOMONAS***

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Proteins synthesized within the chloroplast of *Chlamydomonas reinhardtii* were recovered in granules within vacuoles (Park et al., *J. Phycol.* 35,528-538, 1999). Apoproteins of light-harvesting complexes, synthesized in the cytosol in excess of the capacity of the chloroplast to synthesize chlorophyll, also were recovered in vacuolar granules. The ultrastructure of conventionally and cryo-fixed cells revealed protuberances of the chloroplast envelope outer membrane that contained stroma-like material and also connections between the outer membrane and vacuolar membrane. Transfer of proteins via these structures to vacuoles appeared to be a pathway for degradation of chloroplast

material. Purified granules contained polyphosphate as the major component, which seemed to protect proteins in the vacuole from breakdown. A predominant 70 kDa protein in purified granules accumulated in the cell wall of wild-type cells and in granules of wall-deficient cells, as revealed by immunolocalization. This protein entered vacuoles via small vesicles from Golgi, which probably also introduced degradative enzymes into this compartment. Vacuoles thus apparently serve as lysosome-like structures as well as secretory vesicles. The membranes of the chloroplast envelope in cryo-fixed green cells were tightly appressed except where the inner membrane invaginated or the outer membrane protruded into the cytosol. Whereas conventional fixation showed lack of appression of thylakoid membranes in a chlorophyll *b*-less mutant, these membranes appeared as large grana in images of cryofixed cells. The enhanced images obtained with cryofixation allowed substantial refinement of our knowledge of the ultrastructure of these algal cells.

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**IDENTIFICATION OF A GLYCINE DECARBOXYLASE GENE IN *THALASSIOSIRA WEISSFLOGII*: A PROBE FOR PHOTORESPIRATION IN DIATOMS**

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Diatoms are responsible for a large percentage of CO<sub>2</sub> fixation in the world's oceans. The export of diatom-fixed carbon from the euphotic zone represents a major sink of CO<sub>2</sub> in the global C cycle. Under conditions of high light and high O<sub>2</sub>/CO<sub>2</sub>, carbon fixation may be reduced due to photorespiration. The fixation of O<sub>2</sub> in photorespiration produces low molecular weight metabolites that cannot be used in the Calvin cycle and are either excreted from the cell or inefficiently salvaged in the photorespiratory pathway. One of the key enzymes of photorespiration is glycine decarboxylase (GDC). We have cloned and sequenced a cDNA corresponding to the gene encoding the T-protein of GDC from the centric diatom *Thalassiosira weissflogii*. This cDNA is 1100 bp and its predicted amino acid sequence shows a 45% identity and a 62% homology with the T-protein from higher plants. The first 20 amino acids display characteristics of mitochondrial transit peptides, consistent with the localization of GDC in the mitochondria of higher plants. Using competitive RT-PCR we have demonstrated that transcription of this gene is light-dependent. Cells maintained at constant growth-saturating light conditions have much higher levels of T-protein message than cells kept in the dark for 25 hours. We are currently investigating the effect of other environ-

mental parameters, such as O<sub>2</sub> concentration, on T-protein gene transcription. Ultimately, the goal is to use this gene as a probe for diatom photorespiration in the field. To this end, we are developing degenerate primers and sequencing this gene from other diatom species.

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**TAXONOMIC REASSESSMENT OF THE GENUS *PADINA* (DICTYOTALES, PHAEOPHYTA) IN THE GULF OF CALIFORNIA, MÉXICO**

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*Padina* is one of the most important genera in the coastal zone of the Gulf of California because of their biomass. Eight species have been recorded for the area: *P. tetrastromatica*, *P. durvillaei*, *P. gymnospora*, *P. mexicana*, *P. caulencens*, *P. conrescens*, *P. crispata* y *P. vikersiae* based on taxonomic and floristic studies. Unfortunately, until today, a comparative study of all these species in a modern context was not available. Intensive surveys in 100 localities around the area yield more than 1000 specimens and 500 slides were analyzed and compared with relevant types. Taxonomic evaluations were developed using 16 characters commonly used in the literature. As a result of our analysis, only five characters can discriminate species. Based on character analysis and comparisons with proper type material, only three species can be recognized in the zone: *P. conrescens*, *P. durvillaei* and *P. mexicana*. In the case of *P. crispata*, we conclude that it is a heterotypic synonym for *P. mexicana*. But in the case of *P. gymnospora*, *P. tetrastromatica* and *P. vikersiae*, all previous records represent mis-identifications.

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**SEASONAL AND ZONAL VARIATION IN NITROGEN SOURCE FOR FOUR INTERTIDAL SEAWEEDS FROM NEW ZEALAND**

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Seasonal patterns in nitrogen uptake by four species of intertidal macroalgae (*Stictosiphonia arbuscula*, *Aophlaxa lyallii*, *Scytothamnus australis*, *Xiphophora gladiata*) from Otago in New Zealand's south island were investigated. Field-collected material from species occurring at different shore heights was exposed to inorganic (NO<sup>-3</sup>, NH<sup>+4</sup>) and organic (urea) nitrogen at different concentrations (5µM, 30µM), and the rate of uptake over 30min intervals for 3hr was determined. Active uptake of nitrate was shown by all spe-

cies in both seasons but uptake rates were 2–5 times greater in summer compared to winter. Ammonium uptake was highest in all species regardless of season, indicating a preference for this form of nitrogen. For all species, urea uptake was negligible during winter but represented a substantial proportion of the total nitrogen taken up during summer. Comparison of species in relation to their shore position revealed differences in uptake rates for each nitrogen source. These results suggest that intertidal seaweeds growing at different shore positions employ different strategies for procuring nitrogen in an environment where nutrient supply may be limited.

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***SONDERELLA*, *LEMBERGIA* AND THE NEW TRIBE SONDERELLEAE (RHODOMELACEAE, RHODOPHYTA)**

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The monospecific genus *Sonderella* is endemic to Australia and has been the subject of speculation with regard to its taxonomic position. When the anatomy and reproduction of this genus were comprehensively described by Womersley in 1965, he recommended its inclusion in the Rhodomelaceae and foreshadowed that it would warrant its own tribe. Recent molecular investigation has confirmed its position within the Rhodomelaceae. Classical anatomical investigation of the rare and poorly-known New Zealand endemic genus *Lembergia* has shown that it shares many features with *Sonderella*, including vegetative construction and position and construction of the reproductive structures. Molecular investigations of the 18S rRNA gene support the close relationship of the two genera and their inclusion within the Rhodomelaceae as a separate tribe, the Sonderelleae tribus novum.

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**TAXONOMY OF THE *LENORMANDIA* - *LENORMANDIOPSIS* COMPLEX (RHODOMELACEAE, RHODOPHYTA)**

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The genus *Lenormandia* is composed of nine species from Australia and New Zealand. Some of these are well known, but others are rare, obscure and ill-defined. We have examined material of all described species and found that they fall into two discrete groups that differ in apex morphology and position of reproductive structures. Plants of the first group, con-

taining the type species *L. spectabilis*, have a cleft apex and reproductive structures produced directly on the blade surface, whereas those of the second group have a strongly inrolled apex and produce reproductive structures dorsally on small branchlets which arise either from the margins or the midrib. The groups were also found to form discrete clades on analysis of 18S rRNA sequences. All the members of the first group are endemic to Australia, whereas the second group, designated by the new genus name *Adamsiella*, contains two previously described New Zealand species and a single Australian representative. In addition, two new species are described in this group from New Zealand. Members of the closely related genus *Lenormandiopsis* were also examined and the type species, *L. latifolia*, was found to conform in apex morphology and position of reproductive structures to the genus *Lenormandia*. Accordingly *Lenormandiopsis* has been subsumed within *Lenormandia*. The remaining three members of the former genus *Lenormandiopsis*, however, were found to differ from both the type species and the genus *Lenormandia* and consequently have been transferred to the separate genus *Geraldia*, along with a new species from Geraldton, Western Australia which is designated as the type.

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##### **GLOBAL SYSTEMATIC AND PHYLOGENETIC ANALYSIS OF SARGASSUM IN THE GULF OF MEXICO, CARIBBEAN AND PACIFIC BASIN**

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*Sargassum* is one of the most species-rich genera in the brown algae with over 400 described species worldwide. The bulk of these species occurs in Pacific-Indian ocean waters with only a small portion found on the Atlantic side of the Isthmus of Panama. *Sargassum* also has one of the most subdivided and complex taxonomic systems used within the algae. Systematic distinctions within the genus are further complicated by high rates of phenotypic variability in several key morphological characters. Molecular analyses in such systems should allow testing of systematic concepts while providing insights into speciation and evolutionary patterns. Global molecular phylogenetic analyses using both conserved and variable regions of the Rubisco operon (*rbcL* and *rbcL-IGS-rbcS*) were performed with species from the Gulf of Mexico, Caribbean, and Pacific basin. Results confirm earlier analyses based on *rbcL-IGS-rbcS* from Pacific species at the subgeneric and sectional level while providing additional insights into the systematics and phylogenetics

on a global scale. For example, species east of the Isthmus of Panama form a distinct well-resolved clade within the tropical subgenus. This result in sharp contrast to traditional systematic treatments but provides a window into the evolutionary history of this genus in the Pacific and Atlantic Ocean basins and a possible means to time speciation events.

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##### **MODULATION OF VERY-LONG CHAIN (C<sub>28</sub>) HIGHLY UNSATURATED FATTY ACIDS IN PROROCENTRUM MINIMUM (DINOPHYCEAE) BY SELENIUM**

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Recently, very-long-chain (C<sub>28</sub>) highly unsaturated fatty acids (VLC-HUFA) were identified in seven marine dinoflagellate species (Manour *et al.*, *Phytochemistry*, 1999, 50: 541–548). In general, the proportion of these fatty acids accounted for less than 2.3% of the total fatty acids in these species. As part of a study investigating the modulation of the hemolytic fatty acid 18:5n<sub>3</sub>, cultures of *Prorocentrum minimum* were grown in artificial seawater with varying molarities of sodium selenite (0, 1, 10, and 100 nM). Optimal growth was observed at 1 nM with this media. As expected, the level of 18:5n<sub>3</sub> was modulated by the selenium in the culture medium (7.0 ± 0.2, 14.5 ± 0.6, 7.4 ± 0.8, and 3.9 ± 0.8% of total fatty acid, respectively), with the highest percentage found at 1 nM. Unexpectedly the level of VLC-HUFA (28:8n<sub>3</sub>) increased to 7.3 ± 2.8% at 0 nM sodium selenite, while at all other selenite concentrations the VLC-HUFA was less than 1%. A possible biochemical basis for this finding will be discussed.

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##### **CALCIUM OXALATE CRYSTALS IN THE GREEN ALGA SPIROGYRA SP. (ZYGNE-MATALES, CHLOROPHYTA)**

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Specimens of an unidentified species of the freshwater green alga *Spirogyra* were found to have abundant cruciate cellular inclusions up to 34 micrometers long. A crystalline nature was shown by birefringence in polarized light. Despite their large size and complex shape, these inclusions did not occur free in the large central vacuole. Instead, they were associated with cytoplasmic strands that spanned the space between gyres of the parietal spiral chloroplasts and with strands that suspended the nucleus in a cytoplasmic

embayment of the central vacuole. Some crystals moved directionally along the cytoplasmic strands, and their movement was arrested by cytochalasin B, suggesting that actin microfilaments had a role in crystal movement. Solubility tests showed that the inclusions were composed of calcium oxalate; they dissolved rapidly in weak hydrochloric acid without effervescence, but they were not soluble in concentrated acetic acid or sodium hypochlorite. A colorimetric enzymatic test for oxalate was used to demonstrate microscopically the presence of oxalate and to quantify the amounts. The calcium oxalate crystals were surrounded by a water-soluble organic matrix that retained the shape of the crystal even after demineralization. Scanning electron microscopy was used to examine the morphology of isolated crystals.

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### EXCHANGES OF INFORMATION, ENERGY & MATERIALS IN SYMBIOSES

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Symbiosis is important in the cell and environmental biology of algae. Some examples involving the author and numerous collaborators include: 1) chloroplasts of eukaryotic algae arose from endosymbioses. Plastids are incapable of independent existence; most of the genes of the cyanobacterial photobiont have been lost, and the majority of the rest have been transferred to the nuclear genome. Some of the genes retained by the plastid are those whose transcription is controlled by environmental cues transduced by the organelle. The general trend is for organelle genes to be transferred to the nucleus, escaping plastid redox activities generating mutagenic free radicals; 2) symbioses involving potentially free-living photobionts include marine lichens and sponges with cyanobacterial symbionts. For the lichen, *Lichina*, inorganic carbon acquisition appears to involve inorganic carbon transport by the mycobiont, and for the sponge, *Cymbastella*, the flagellar activity of the sponge is probably important for inorganic carbon supply to the photobiont; 3) the Australasian fucalcan, *Notheia*, is an obligate epiphyte on the fucalcan, *Hormosira* and *Xiphophora*; the four species involved all contain the hexitol, altritol. *Notheia anomala* is known to be phylogenetically-distant from the other five altritol-containing species. Can *Notheia* synthesize altritol, or is it obtained from the phorophyte?; 4) Sacoglossan gastropods retain kleptoplastids (not strictly symbionts) from ulvophycean (or rhodophycean) marine algae. Analyses of the natural abundance of stable carbon isotopes suggest significant contribution of kleptoplastid photosynthesis to the carbon and energy budget of the mollusks.

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### PATTERNS AND CONSEQUENCES OF SPORE DISPERSAL IN THE GIANT KELP, *MACROCYSTIS PYRIFERA*

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We investigated patterns of spore dispersal in the giant kelp *Macrocystis pyrifera* by collecting 80 independent measurements of spore dispersal from isolated individuals and isolated groups of individuals over a two-year period. Our results indicate that giant kelp spores routinely disperse both short (i.e. a couple meters) and long (i.e. hundreds to thousands of meters) distances depending on the oceanographic conditions. One consequence of spore dispersal over short distances is self-fertilization (i.e., fertilization between male and female gametophytes derived from the same sporophyte). Field experiments designed to test the effects of self-fertilization on lifetime fitness in *Macrocystis* revealed significant inbreeding depression. Birth rates in self-fertilized populations were ca. 50% of those produced from outcrossing, which lead to significant differences in cohort size that persisted up through the adult stage. In contrast to outcrossed populations, very few individuals produced from selfing became reproductively mature, and those that did were significantly less fecund than outcrossed individuals. By contrast, long-range dispersal of spores leads to increased rates of outcrossing. However, long-range dispersal is typically accompanied by massive dilution of spores, leading to low densities of spore settlement. Sparse spore settlement decreases the overall chance of fertilization in the microscopic gametophyte generation thereby reducing the potential for colonization of the macroscopic sporophyte stage. Large population size of adult sporophytes coupled with the synchronous release of spores in response to environmental cues can help offset the effects of spore dilution and extend the distances over which giant kelp is able to colonize.

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### THE COMPOSITION AND TOXICITY OF DINOFLAGELLATE BLOOMS IN THE SALTON SEA, CALIFORNIA

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Phytoplankton blooms have been implicated in mortality events of diverse groups of organisms including fish, birds and humans. About 300 species have been reported to form "red tides," or surface discolorations due to high densities, but only 60–80 of these species produce harmful blooms. In marine systems, dinoflagellates account for 75% of all harmful algal bloom species. The Salton Sea is a large saline lake located in southeastern California, USA. The lake is eutrophic largely because it is in a closed basin and receives most of its input from agricultural and municipal wastewaters. Dinoflagellates comprise a significant portion of the phytoplankton biomass, particularly in winter, often resulting in "red" or "brown" tides. To date, 16 species of dinoflagellates have been identified from the Salton Sea, and many other unidentified forms have also been documented. In 1992, 150,000 eared grebes were found dead over a period of several months at the Salton Sea. This mortality event was among the largest of any bird species. The principal cause remains unknown, but algal toxins were suspected. A survey of the composition and toxicity of algal blooms was undertaken in 1999, and we report results from blooms where dinoflagellates dominated. Dominant species included *Gonyaulax grindleyi*, *Gymnodinium* spp., *Gyrodinium uncatenum*, *Heterocapsa niei*, and an unidentified scrippsielloid. Although most samples showed activity in a brine shrimp lethality assay, all were negative in a mouse bioassay. This evidence suggests that toxins from dinoflagellate blooms in the Salton Sea are not responsible for eared grebe mortality events.

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#### **SPECIES-SPECIFIC RESPONSE IN ALKALINE PHOSPHATASE ACTIVITY OF FRESHWATER PHYTOPLANKTON IN A NUTRIENT ENRICHMENT EXPERIMENT**

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A new method was utilized to study species-specific responses of phytoplankton to phosphorus limitation in a nutrient enrichment experiment. A substrate, ELF, produces a fluorescent precipitate at the sites of alkaline phosphatase (AP), which makes it possible to visually detect phosphorus (P) limitation in individual cells of multiple species. Lake water was incubated in the laboratory to induce nitrogen (N) or P limitation. Initially, little or no ELF labeling was observed for any of the phytoplankton species, indicating a general

lack of P limitation. This observation was supported by low bulk AP activity in the initial field samples. During the experiment, several chlorophyte taxa (*Coelastrum*, *Eudorina*, a solitary spiny coccoid) were driven to P limitation, as evidenced by a high percentage of cells displaying ELF labeling when inorganic N was added. Taxa such as *Actinastrum* and *Dictyosphaerium*, on the contrary, were never P limited. Little or no ELF was observed in cyanobacterial species, suggesting that P limitation was not achieved in these organisms. Using traditional bulk AP activity, significantly higher levels of AP activity were observed in treatments with inorganic N additions, compared to those with phosphate additions. ELF labeling generally followed the trend of bulk AP, except in species that did not dominate the biomass. Finally, we noted that all species observed were ELF labeled at least on one occasion, except for fragile flagellates which did not withstand the labeling procedure.

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#### **RANGE EXTENSION OF *CUTLERIA HANCOCKII* (CUTLERIALES; PHAEOPHYTA) TO THE SOUTHWESTERN GULF OF CALIFORNIA, MÉXICO**

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A range extension of *Cutleria hancockii* Dawson to the southern portion of the Gulf of California is presented based on collections in Concepción Bay, La Paz Bay and Muertos Bay between March, 1996 and May, 2000. Mature gametangial and sporangial plants were collected in several localities in the low intertidal zone and in subtidal areas. The present records represent a range extension of 500 km for the species with the new southern limit in Punta Perico in Los Muertos Bay. The plants mostly were observed in low intertidal-shallow subtidal areas associated with *Sargassum* forest, but also were observed at depths of 30 ft. Based on analysis of the basic morphological and anatomical features of the plants, significant differences within geographic position and depth distribution were noted. Our data suggest that the temperature may be the determining factor in the distribution of this species.

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#### **DEMOGRAPHY OF FRONDS OF *SARGASSUM LAPAZEANUM* FROM SOUTHERN BAJA CALIFORNIA, MEXICO, DURING EARLY STAGES**

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In terms of biomass, *Sargassum lapazeanum* (Phaeophyceae, Fucales) is one of the most important seaweeds in La Paz Bay, on the southeastern coast of the Baja California Peninsula, Mexico. This species can be found year-round in shallow subtidal sites. Standing biomass is lowest between fall and winter and highest between spring and summer. We are currently studying the annual demography of fronds as a necessary step to understanding the mechanisms of population regulation. Work is being done on a bed of about 90 m in length and three m in width. At this stage of abstract submission, we can report results for winter only. Random samples were collected in February and in March 2000 to estimate stand biomass, frond density, and size (frond length) structure. In addition, we labeled fronds with numbered plastic tags to estimate their rates of growth and of mortality during this period. Rates of recruitment were estimated from a combined analysis of the above. In February, fronds were all shorter than 6.5 cm. Between February and March, mean total frond density increased from 122 to 776 fronds  $m^{-2}$  ( $n = 30$  quadrats). The mean recruitment rate was 667 fronds  $m^{-2}$ , the mean growth rate was 0.5 cm  $day^{-1}$  ( $n = 60$  fronds), and the mean mortality rate was 43 fronds  $m^{-2}$ . From last year's preliminary observations, we expect to observe peaks of biomass and of reproduction in late spring, followed by negative growth rates and high mortality rates during summer.

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#### EVOLUTION OF BIOLUMINESCENCE IN MARINE DINOFLAGELLATES

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Bioluminescence is broadly distributed in marine dinoflagellates and has been intensively studied in *Lincolodinium (Gonyaulax) polyedra*. In this species, bioluminescence is regulated in a circadian fashion; the enzyme (luciferase) and the luciferin (substrate)-binding protein are synthesized and degraded on a daily basis. Synthesis of both proteins is regulated at the level of translation. The *L. polyedra* luciferase gene is composed of three contiguous domains that are greater than 75% identical at the nucleic acid level. Possible explanations for the high degree of sequence conservation include: (1) the domains evolved through a recent duplication event; (2) the sequence similarity is maintained by a molecular process such as gene conversion; or (3) there is a functional role associated with the primary nucleic acid sequence, such as in the translational regulation of luciferase expression. The phylogenetic relationship of dinoflagellates predicted from 18S rDNA genes provides a framework for examining the molecular evolution of the regulation of luciferase expression and of genes encoding luciferase and the luciferin-binding protein. In particular, we are examining the evolution of the

circadian rhythm of bioluminescence and of luciferase abundance, the presence/absence of the luciferin-binding protein, and the molecular structure of the luciferase gene. We anticipate that this approach will distinguish between regions of the luciferase molecule that are conserved for enzyme function versus those concerned with the regulation of protein expression. In addition, it will provide insight into the evolution of the regulatory processes and pathways.

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#### ANALYSIS OF NUCLEAR MUTANTS OF CHLAMYDOMONAS DEFICIENT IN THE ACCUMULATION OF SPECIFIC CHLOROPLAST RNAS

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Genetic analysis has revealed that the accumulation of several chloroplast mRNAs of the green alga *Chlamydomonas reinhardtii* requires specific nucleus-encoded functions. We have characterized three nuclear mutants, *nac2*, 222E and F24 which lack *psbD*, *psbB* and *psaB* mRNA, respectively. In each case we have shown that the target of the nucleus-encoded function affected in these mutants is the 5' untranslated region of the corresponding mRNA (3, 4, 5). We have cloned the nuclear *Nac2* and *Mbb1* genes by rescue of the mutants which are deficient in the accumulation of the mRNAs of *psbD* and the *psbB/psbT/psbH* chloroplast transcription unit. *Nac2* encodes a hydrophilic polypeptide of 1385 amino acids with 9 tetratricopeptide repeats (TPR) in its C-terminal half whereas *Mbb1* encodes a polypeptide of 662 amino acids which also contains 9 TPRs. Both proteins contain a putative chloroplast transit peptide at their N-terminal end. They have been tagged with the HA epitope and localized in the stromal compartment of the chloroplast. *Nac2* and *Mbb1* are part of a high molecular weight complex of 500 and 250 kDa, respectively, which is associated with RNA in the case of *Nac2*. A change of a conserved Ala residue of the fourth TPR motif by site-directed mutagenesis completely abrogates *Nac2* function indicating that this TPR is important for *psbD* mRNA stability, processing and/or translation.

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#### PRELIMINARY FLUORESCENCE AND ULTRASTRUCTURAL OBSERVATIONS IN *DICTYOSPHAERIUM PULCHELLUM* (CHLOROCOC-CALES, CHLOROPHYCEAE)

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*Dictyosphaerium pulchellum* is a common freshwater phytoplankton. After cell division, autospores remain attached to each other in a characteristic fashion through the remnants of the partially hydrolyzed mother cell walls (tracts), originating up to 64-celled colonies immersed in abundant mucilage. Staining with ruthenium red and toluidine blue revealed mucilage acid properties, probably due to uronic acids in polysaccharides. Tracts and cell walls were Periodic Acid-Schiff positive and showed fluorescence with calcofluor white, indicating the presence of fibrillar polysaccharides of the type cellulose and beta-mannans. Intense fluorescence with berberine suggested some hydrophobic coating of fibrillar polysaccharides. Though cell walls proved to be acetolysis resistant, no secondary fluorescence was observed with primuline, suggesting that the hydrophobic polymer in the cell wall differed from algaenan. Ultrastructural observations revealed no trilaminar structure in the cell wall, but ruthenium red stained sections exhibited a "zipper-like" band in the outermost cell wall layer both in autospores and mother walls. The cells have a large cup-shaped chloroplast containing a single pyrenoid with the matrix traversed by double thylakoids and circled by starch plates. A lobulated nucleus occupied the concavity of the chloroplast. Dictyosomes appeared closely related with the nuclear envelope. Several vacuoles scattered in the cytoplasm and close to the nucleus showed electron dense content, which could also be observed in the periplasmic space. Fluorescent dye quinacrine also showed active vesicle traffic.

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#### PROTEIN EXPRESSION DURING HEAT STRESS IN THERMO-INTOLERANT AND THERMO-TOLERANT DIATOMS

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To better understand how diatoms are capable of responding to environmental stress, protein expression during heat treatment of a thermo-intolerant (*Phaeodactylum tricorutum*) and thermo-tolerant (*Chaetoceros muelleri*) diatom (Chrysophyta) was investigated. The stress response is a universal and conserved mechanism of cell survival to unfavorable conditions. Typically, a 10 to 15° C temperature elevation above cell growth optimal causes constitutively expressed pro-

teins to decrease and heat shock proteins (HSPs) to increase. HSPs are categorized by molecular weight among five classes with each apparently specialized for a particular function that enhances cell survival. One-dimensional SDS-PAGE of diatoms subjected to heat treatment revealed that *P. tricorutum* exhibited a typical stress response, but *C. muelleri* did not exhibit a characteristic response even at a greatly elevated temperature (50° C). This result was confirmed by total soluble protein assays. *Chaetoceros muelleri* may contain higher basal levels of HSPs than *P. tricorutum* allowing *C. muelleri* to better tolerate elevated temperatures. Western blot analysis using pea HSP70 (70 kDa) antisera of heat-treated *P. tricorutum* and *C. muelleri* validated the hypothesis that thermo-tolerant cells contain higher levels of constitutively expressed HSPs. Two-dimensional gel electrophoresis of heat-treated cells indicate that the small HSPs (17–30 kDa) played a role in the stress response similar to that found in vascular plants. Ongoing work is focused on the manipulation of the stress response through over-expression of key hsp genes.

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#### REVISED CLASSIFICATION OF THE GENUS PACHYMENIA BASED ON MORPHOLOGICAL AND MOLECULAR CHARACTERS

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Taxonomic discrimination in the genus *Pachymenia* (Rhodophyta) in New Zealand is based primarily on phenotypic characters of the thallus. The taxonomic problems raised by this classification method are due to highly variable thallus characters such as blade thickness, blade width, degree of thallus branching, and variation in anatomical characters. Delineation of species is further complicated by a lack of adequate knowledge about the responses of phenotype to environmental variation. There are currently three species recognized in this genus that are endemic to New Zealand: a prostrate species *P. crassa*, and two erect species, *P. laciniata* and *P. lusoria*. In this study, two approaches are used to investigate the current delineation of these species. Morphological and anatomical characters of field collected material and herbarium specimens from throughout the species' distributional ranges were quantified. Multivariate analyses were used to identify discrete phenotypic groups. Species relationships were further analyzed by quantify-

ing the variation found within the internal transcribed spacer region (ITS) of the nuclear ribosomal DNA. The results obtained from both approaches will be discussed with regards to possible re-classification of species relationships within this genus. We suggest that the two erect species should be merged, and the currently recognized *P. lusoria* should be separated into at least two taxonomic groups.

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**COALESCENCE VERSUS COMPETITION: FIELD AND LABORATORY STUDIES OF INTRA- AND INTERSPECIFIC ENCOUNTERS AMONG COALESCING SEAWEEDS**

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Classical ecological theory predicts that whenever growing individuals share a common and limiting resource, such as substratum in mid-intertidal and shallow subtidal habitats, preemptive competition will occur determining species abundance and distribution patterns. However, conspecificity of several ecologically dominant Rhodophyta may coalesce when grown in laboratory cultures. The extent at which intraspecific coalescence occurs in the field and whether the process may also happens during interspecific encounters remain to be determined. If intra- and interspecific coalescence effectively occurs, then coexistence through coalescence rises as an alternative to competition among red-algal dominated intertidal and shallow subtidal communities. Populations of *Mazzaella laminarioides* and *Nothogenia fastigiata* living in mid-intertidal, semi-exposed rocky habitats in Central Chile are being used to test the above ideas. Intra- and interspecific encounters occur in the field throughout the year. Coalescence does occur among conspecific partners but it has not been detected in interspecific encounters. Rather, a thick interface of compressed cells, necrotic tissues and cyanobacterial nodules is formed between the two contacting partners. In addition, observations of laboratory cultures indicate that spore germination, germling survival and differentiation of erect axes in bispecific cultures may be reduced when compared to single-species controls. Interspecific differences in growth and differentiation rates appear as the mechanisms explaining a lack of coalescence and negative effects during interspecific contacts. On the other hand, the existence of conspecific coalescence in the field suggests this process should be considered as a real alternative to intraspecific competition among coalescing Rhodophyta.

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**PREDAEA LUCESCENS SP. NOV., A NEW COOL-TEMPERATE AUSTRALIAN SPECIES WITH UNIQUE, UNDIFFERENTIATED NUTRITIVE FILAMENTS**

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The red algal genus *Predaea* (Schizymeniaceae, Gigartinales) includes approximately a dozen species of tropical and subtropical distribution. Plants are gelatinous in consistency, and the carpogonial branches and auxiliary cells are widely separated in female gametophytes (non-procarpy). *Predaea* is distinguished from other red algal genera in possessing clusters of small rounded nutritive cells that are borne on the one proximal and distal two cells directly attached to the auxiliary cell. We describe a new species from cool-temperate (10–18° C yearly range) southeastern Australia that, unlike the tropical and subtropical species, lacks the typical gelatinous texture and has dwarf vegetative filaments of normally contoured cells associated with the auxiliary cell rather than small clusters of spherical nutritive cells. Anatomical and molecular data are combined to postulate the phylogenetic affinities of the new species to others of the genus, to highlight relationships of *Predaea* to other genera of the Schizymeniaceae, and to consider this family relative to others now placed in the Gigartinales.

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**A CANDID ASSESSMENT OF SYSTEMATICS IN THE LAMINARIALES: MUDDLED MORPHOLOGIES AND SKETCHY SEQUENCES**

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Algae of the Alariaceae, Laminariaceae and Lessoniaceae are the largest, most complex, and, arguably, most fascinating of the seaweeds. It is, therefore, a profound paradox that these species remain in taxonomic chaos despite the contemporary emphases on systematics. Setchell and Gardner established the classification system in 1925, recognizing these families on the basis of clear morphological features. At that time, however, they acknowledged that some species had features consistent with placement in two families, or that obscured logical placement in any of the families. Ironically, the problems noted by Setchell and Gardner have been ignored and the system has become entrenched in kelp literature. Initial molecular studies highlighted the shortcomings noted by Setchell and Gardner, and further indicated that little of the morphology-based system was natural. It was obvi-

ous that the diagnostic morphological features, presence or absence of sporophylls and ontogenetic splitting, were 'noisy' being gained and lost independently many times in kelp evolution. Despite the insights of the initial molecular studies, they had limitations and key relationships remained unresolved. The investigations used the Internal Transcribed Spacers (ITS) of the ribosomal cistron that have phylogenetic limitations owing to their short length, which is exacerbated by the many variable regions that can't be aligned confidently and must be removed prior to phylogenetic analyses. Many molecular publications have appeared subsequent to the first ITS results, and their contribution towards elucidating kelp phylogeny will be assessed. Current investigations using the Large Subunit ribosomal DNA (LSU) for kelp systematics will also be discussed.

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### **INTRODUCED MACROALGAE IN THE AUSTRALIAN REGION: CURRENT STATE OF KNOWLEDGE**

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Introductions of non-native macroalgae and the subsequent displacement of native species are globally becoming more frequent. The algal genera *Undaria*, *Sargassum*, *Caulerpa* and *Codium* have been identified as being particularly invasive. An overview on the present knowledge on macroalgal introductions in the Australian region is presented and options for management are discussed, mostly using examples from studies on the introduced Japanese kelp, *Undaria pinnatifida*. *Undaria pinnatifida* was first detected in Tasmania, Australia in the early 1980's. Since then, its range has expanded despite eradication efforts. Long distance jumps appear to be the major mode of spread of *U. pinnatifida* in Tasmania. Studies are underway to distinguish the relative importance of spore dispersal, drift of adult plants and anthropogenic factors in spreading this invasive kelp. Although information on the real impacts of *U. pinnatifida* and other introduced macroalgae is sparse, the development of management and control strategies is of vital importance to prevent further spread and translocation of these "pest" species.

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### **CONTRASTING LIFE HISTORIES AND DEMOGRAPHIES OF LAMINARIAN AND FUCOID ALGAE**

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Many species of large brown algae co-occur along similar shores and depths and seem to share similar ecological characteristics in terms of habitat-formation, providing nursery and feeding habitats for reef fish, vertical structure of habitats within the water column and biomass production. However, the dominant species have very different life histories. Laminarians have a heteromorphic alternation of generations, while fucaleans have an animal-like life history with more direct development. Despite these differences, there seems to be no obvious relationship between life history and ecological function. Laminarians and fucaleans seem generally to have very different demographies, with quite disparate features such as propagule size and number, early survival, growth rates, and densities of plants required for a closed canopy. Furthermore, few laminarians occur in the intertidal zone. Here, I will compare some species in these groups for which we know something about life history and ecology in New Zealand and the USA. Although it is necessary to understand life histories of individual species to understand their demography and ecological functions, this is not sufficient in itself to predict such roles and functions.

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### **"SCATTERED CP-NUCLEOIDS" IN DIATOMS EXPLAINED: BACTERIA - INSIDE THE ENDOPLASMIC RETICULUM - PIERCE THE PLASTIDS OF PINNULARIA**

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Geitler's (1937) observation on the regular perforation of the chloroplasts of *Pinnularia nobilis* and the lack of a typical pyrenoid is supported using cytochemistry, and fluorescence & electron microscopy. Chloroplast cavities and channels harbour bacteria, their DNA elicit DAPI-fluorescence. Previous speculations on the DNA-positive, achromatic dots in the plastids of several *Pinnularia* species being "scattered chloroplast nucleoids," and the consequent separation of diatom plastids in "primitive" and "advanced" types, are thus refuted. Bacteria are rod-shaped and as suggested by their TEM-profiles gram-negative proteobacteria. They occur inside the endoplasmic reticulum throughout the cell, in interphase prevailing in the space between the periplastidial membrane (PPM) and the CER membrane. Clusters of bacteria inside ER-cisternae near the nucleus in preprophase, and near the new plasmalemma after cleavage, indicates a cell-cycle dependent translocation within the diatom. Cavities in the plastids seem to be created by a combination of mechanical and chemical forces, in the sequence: alignment, attachment, deformation and lysis, initially without an obvious disruption of the PPM and the chloroplast-envelope. Our future goal is to identify the bacteria and their metabolic function

in the hereditary association with the plastids in *Pinularia*. Their intracellular, but extraplasmatic, location may reflect an ancestral constellation, and the apparent worldwide distribution may substantiate this view.

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##### THE EFFECT OF SEAWEED DIETS ON GROWTH OF GREEN ABALONE, *HALIOTIS FULGENS*, FROM BAJA CALIFORNIA, MEXICO

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Two abalone species: green *Haliotis fulgens* and yellow *Halioti corrugata* represent nearly 97% of the total production in the Mexican abalone fishery. It has been assumed that abalone feed on the kelp algae *Macrocystis pyrifera*. Regional hatcheries use this species as a main source of natural food. *M. pyrifera* does not occur at the southern limit of the distribution of abalone species along the Baja California Peninsula. In this study, growth rates of juveniles *H. fulgens*, 17.3 ± 2.2 mm shell length and 0.4 ± 0.2 g body weight, were evaluated. Juveniles were fed with common species in the benthic environments inhabited by abalone along the western coast of Baja California during 191 days. Three diets were based on algae: palm kelp, *Eisenia arborea*, giant kelp, *M. pyrifera* and *Gelidium robustum*, and one on seagrass, *Phyllospadix torreyi*. Shell length and body growth rates varied between 21.5 µm day<sup>-1</sup> and 2.2 mg day<sup>-1</sup> for *E. arborea* and between 45.9 µm day<sup>-1</sup> and 6.7 mg day<sup>-1</sup> for *M. pyrifera*. Higher specific growth rates (SGR) in length and weight were determined for *M. pyrifera*: 0.2% and 0.7% day<sup>-1</sup>. Significant differences between values of juveniles fed *M. pyrifera* with the rest of the diets were found. The highest mortality (21%) was in juveniles fed the red algae *G. robustum*.

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##### A PROPOSAL FOR A NEW RED ALGAL ORDER, THE THOREALES

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The freshwater red algal genera *Nemalionopsis* and *Thorea* are currently classified in the family Thoreaceae of the Batrachospermales. However, these two genera differ from other members of the order in having multiaxial thalli. The phylogenetic relationships of the genera were investigated using a combination of DNA

sequence analysis (*rbcL* and 18S rRNA genes) and transmission electron microscopy. In both gene trees, *Nemalionopsis* and *Thorea* form well supported clades (87–100% bootstrap values) separate from other members of the Batrachospermales as well as taxa from the Acrochaetiales, Palmariales, and Nematiales. In addition, analysis of the secondary structure of the 18S rRNA gene in *Nemalionopsis* and *Thorea* reveals an additional helix, which is not present in any of the other taxa within the Rhodophyta. Pit plugs of the Thoreaceae also differ from those of other members of the Batrachospermales in not having an outer cap layer that is not universally domed. Based on these findings, it is proposed that *Nemalionopsis* and *Thorea* represent a distinct taxonomic group that should be recognized as a new order, the Thoreales.

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##### SYSTEMATIC EVALUATION OF THE GENUS *HILDENBRANDIA* (RHODOPHYTA): A SYNTHESIS OF TECHNIQUES

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The evolutionary relationships among members of the red algal genus *Hildenbrandia* have not been well understood for several reasons. For example, the genus contains both marine and freshwater representatives, all of which are non-calcified and crustose, and few have definitive morphological characters for classification. *Hildenbrandia* is also assumed to be completely asexual (reproduction by tetrasporangia in marine forms and by gemmae in freshwater populations), and characters of the female gametangial system and post-fertilization structures are not available for comparative studies. Currently there are 14 marine and five freshwater species and infraspecific taxa recognized within the genus. We used phylogenetic analyses (parsimony, distance and maximum likelihood) of DNA sequences of commonly employed genes (*rbcL* and 18S rRNA) to examine the evolutionary relationships among representatives of many of these taxa. In addition, we employed morphometrics (principal co-ordinates and cluster analyses) of several measured characters of these same representatives, as well as all available type specimens, to determine the number of morphologically-delimited entities within the genus. Thus far our results indicate that some characters traditionally used to distinguish species of *Hildenbrandia*, such as tetrasporangial division pattern, may not be useful in some cases, and a revision of the taxonomy of the genus will be necessary. Although the marine and freshwater species of *Hildenbrandia* appear to be well separated in our molecular analyses of European specimens, this trend was not observed for North American specimens. High se-

quence divergence values were calculated for both the *rbcL* and 18S rRNA genes of *Hildenbrandia*, compared to other red algal genera.

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**GEOGRAPHICAL CORRELATION OF MORPHOLOGICAL VARIATION IN *SILVETIA COMPRESSA* (FUCACEAE, FUCALES, PHAEOPHYCEAE)**

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*Silvetia compressa* (J. Agardh) Serrão et al. is a common member of the upper intertidal furoid community on the Pacific coast of America from Humboldt County, California, to Punta Baja, Baja California, Mexico. A relatively narrow range of morphological variability is exhibited by most mainland populations, regardless of latitude, but some mainland populations and all insular populations participate in a complex pattern that we have attempted to analyze. A few populations on the Monterey Peninsula in which the fronds are atypically delicate were described by Setchell & Gardner as f. *gracilis*, to which was assigned a population from Santa Catalina Island. After comparing populations from various parts of the range of the species, including all of the Channel Islands, we conclude that two subspecies may be recognized. In subsp. *compressa*, which includes f. *gracilis* as a growth form and occurs chiefly on the mainland, the frond is robust with long tapered receptacles. In the variant subspecies, which is chiefly insular but also occurs on the coast of northern Baja California, the typical frond has slender axes as in f. *gracilis*, but is more densely branched and has short ellipsoidal receptacles. Comparison of nucleotide sequences from the ITS regions of rDNA revealed an identical pattern for subsp. *compressa* from Baja California and central California, including populations assignable to f. *gracilis*. By contrast, the pattern for the variant subspecies differed by 2 bp (0.3%) from that of subsp. *compressa*.

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**THE DISTRIBUTION AND ABUNDANCE OF DEEPWATER MACROALGAE IN CENTRAL CALIFORNIA**

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Deepwater macroalgal assemblages (DWAAS) were sampled from 30m to their lower depth limits at 4 locations in central California using enriched air Nitrox and the ROV Ventana. The lowest observed depth limits were 78 m for nongeniculate (encrusting) coralline algae, 58 m for non-calcified red algae (i.e. *Maripelta rotata*), 40 m for foliose brown algae (i.e. *Desmarestia tabacoides*), and 37 m for kelps (i.e. *Pleurophyucus gardneri*). Green algae, such as *Derbesia marina* and an undescribed deep water species of *Codium*, were observed at 30m, while large patches of an unknown green or blue-green algal film of coccoid cells were found from 45 to 48 m. DWAAS zonation was generally similar to that in southern California, with subtle differences in the zonation of foliose red algae and the occurrence of an unknown Chlorophyta or Cyanophyta from 45–49m. Twenty four species of algae (13 Rhodophyta, 11 Phaeophyta) were sampled during the percent cover analysis at 30 m. Nongeniculate corallines varied from 53% to 91% cover depending on location, while geniculate corallines (i.e. *Bossiella schmittii*) averaged ca. 25% cover. *Pleurophyucus gardneri*, a stipitate kelp previously described as “rare” in central California, was the most abundant brown alga, averaging from 2.68 to 4.8 individuals m<sup>-2</sup>. Algal abundance (percent cover and density), zonation, and light levels at the lower algal depth limits were similar between sites and locations, suggesting that the environment of deep water algal populations in this region is less variable than that of the shallow water kelp forest communities above them.

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**POPULATION PARAMETERS AND PHOTOSYNTHETIC CAPABILITIES OF CORALLINE RHODOLITHS: BIOGEOGRAPHIC IMPLICATIONS**

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Rhodoliths are bed forming, unattached coralline algae that incorporate large quantities of carbonate into their thalli in oceans worldwide. *Lithophyllum margaritae* and *Neogoniolithon tricotonum* are common Rhodoliths from the Gulf of California, however, little is known about their biogeographic distribution, and their ecological and physiological characteristics. As a

consequence, the objective of this study was to define the temporal population dynamics, growth rates and photosynthetic capabilities of *L. margaritae* and *N. tricotomun* from the Gulf of California. The Gulf of California is characterized by annual temperature fluctuations that can exceed 20° C. Rhodolith beds exceeding one km in length were observed from the intertidal zone to a depth of approximately 20 m at the southern Gulf. Percent cover estimates for spherical unattached individuals of *L. margaritae* and *N. tricotomun* ranged from 10 to 100%. Growth rates were determined by staining the rhodoliths with Alizarine red and evaluating the linear increment of calcium carbonate after an incubation period in the field. Annual growth rates averaged 1.8 mm year<sup>-1</sup> in *L. margaritae* and 3.4 mm year<sup>-1</sup> in *N. tricotomun*, however, growth rates varied seasonally. The photosynthetic response of both species as a function of temperature was evaluated in the laboratory from 10° to 30° C. Maximum net photosynthetic rates peaked at approximately 25 to 30° C in both species, suggesting that maximum growth rates occur during the warmest months of the year. These results lead to a better understanding of the biogeographic ranges of subtidal coralline algae.

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### SYMBIOTIC ORIGIN OF A NOVEL ACTIN GENE IN THE CRYPTOPHYTE, *PYREMONAS HELGOLANDII*

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Cryptophytes are photosynthetic protists that have acquired their plastids through the secondary symbiotic uptake of a red alga. A remarkable feature of cryptophytes is that they maintain a reduced form of the red algal nucleus, the nucleomorph, between the second and third plastid membranes (periplastidial compartment, PC). The nucleomorph is thought to be a transition state in the evolution of secondary plastids with this genome ultimately being lost (e.g., as in heterokonts, haptophytes, euglenophytes) when photosynthesis comes under full control of the "host" nucleus. For this to happen, all genes for plastid function must be transferred from the nucleomorph to the nucleus. In this regard, it is generally assumed that nucleomorph genes with functions unrelated to plastid or PC maintenance are lost. Surprisingly, we show here the existence of a novel type of actin gene in the host nucleus of the cryptophyte, *Pyrenomonas helgolandii*, that has originated from the nucleomorph genome of the symbiont. Our results demonstrate for the first time that secondary symbionts can contribute genes to the host lineage that are unrelated to plastid func-

tion. These genes are akin to the products of gene duplication and provide a source of evolutionary novelty that could significantly increase the genetic diversity of the host lineage. We postulate that this may be a common phenomenon in algae containing secondary plastids that has yet to be fully appreciated due to a dearth of evolutionary studies of nuclear genes in these taxa.

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### THE EVOLUTION OF RNA POLYMERASE II IN RED ALGAE

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Whether red algae are the sister group to green plants has been an intriguing question for over a century. We are investigating both the evolutionary and functional significance of differences in DNA-dependent RNA polymerase II between rhodophytes and other eukaryotic organisms. Results of both phylogenetic and genetic complementation studies are consistent with an origin of the Rhodophyta that was independent of the common ancestor of green plants, animals, fungi and several related protistan groups. Examination of long-branch attraction among Pol II gene sequences indicates that this separate emergence of red algae is not the result of an artifactual grouping of rhodophytes with more rapidly evolving taxa. The absence in red algal pol II largest subunits of a canonical C-terminal domain (CTD), an essential component of mRNA transcription in plants, animals and fungi, further supports the proposition that red algae diverged independently of these groups, and before CTD function was incorporated fully into Pol II function. Evolutionary complementation, that is, in vivo replacement of the wild-type CTD from yeast with RPB1 C-terminal sequences from a red alga, further supports the hypothesis that fundamental differences exist in Pol II function between red algae and organisms with a CTD-based mRNA transcription cycle.

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### BIOCHEMISTRY OF SILICA BIOMINERALIZATION IN DIATOMS

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Diatoms are well known for the intricate patterns of their silica-based cell walls. The complex structures of diatom cell walls are species specific and become precisely reproduced during each cell division cycle, indicating a genetic control of silica biomineralization. Therefore, the formation of the diatom cell wall has been regarded as a paradigm for controlled production of nanostructured silica. However, the mecha-



nisms allowing biosilicification to proceed at ambient temperature at high rates have remained enigmatic. Recently, we have shown that a set of highly cationic peptides (called silaffins) isolated from *Cylindrotheca fusiformis* shells are able to generate networks of silica nanospheres within seconds when added to a solution of silicic acid. Different silaffin species produce different morphologies of the precipitated silica. Silaffins contain covalently modified Lys-Lys elements. One of these lysine residues bears a novel type of protein modification, a polyamine consisting of 6–11 repeats of the N-methyl-propylamine unit. In addition to the silaffins, additional polyamine-containing substances have been isolated from a number of diatom species that may be involved in the control of biosilica morphology. Scanning electron microscopic analysis of diatom shells isolated in statu nascendi provide insights into the processes of pattern formation in biosilica. A model will be discussed that explains production of nanostructured biosilica in diatoms on the basis of these experimental results.

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#### EFFECTS OF INCREASING UV-B RADIATION DUE TO OZONE DEPLETION ON PHOTOSYNTHESIS OF ANTARCTIC CYANOBACTERIA

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Ground level ultraviolet-B (UV-B; 290–320 nm) fluxes in Antarctica have been increasing due to stratospheric ozone depletion. Although mat-forming cyanobacteria are major component of freshwater algal biomass in Antarctica, little is known about their response to increasing ultraviolet radiation (UVR). The present study evaluated the sensitivity to UVR of two strains of mat-forming cyanobacteria with different cell size, *Phormidium murrayi* (6.0 x 3.2 µm) and *Schizothrix calcicola* (2.2 x 2.3 µm). Cyanobacterial photosynthesis was measured under different UV spectral quality and quantity achieved by polychromatic filters with different cutoff wavelengths and neutral density screens. The productivity and irradiance data were used to generate biological weighting functions (BWF) for the assessment of UV inhibition on photosynthesis. The kinetics of UV inhibition, as determined by PAM fluorometry, differed between the two species so that inhibition of *P. murrayi* and *S. calcicola* were modeled based on UV-irradiance and cumulative exposure, respectively. After a one hour exposure, BWF's did not differ between the two isolates of cyanobacteria despite their differences in cell size. To evaluate the negative impact of increased UV-B exposure due to ozone depletion on cyanobacteria, the BWF's were applied to two solar spectra obtained from McMurdo Station, one on a day when the ozone

hole was prominent ( $O_3 = 170$  Dobson units;  $DU = 10 \cdot 3 \text{ cm } O_3$ ), and the other on a day with high ozone concentration ( $O_3 = 328 \text{ DU}$ ). The decrease in ozone level would reduce productivity by 3–8%. Seasonal variation of UVR has a bigger impact on cyanobacterial productivity than ozone depletion.

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#### ANALYSIS OF PORPHYRA RBCL DEMONSTRATES MULTIPLE MIGRATIONS OCCURRED BETWEEN THE NORTH ATLANTIC AND NORTH PACIFIC.

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The taxonomic and evolutionary relationships between *Porphyra* of the boreal North Pacific (NP) and North Atlantic (NA) are being examined using near full length sequences of *rbcl* and the *rbcl* *rbcs* spacer (~1420 bp). Phylogenetic analysis of >20 recognized species produced clusters containing both NP and NA species: *Porphyra carolinensis* (NA) and *Porphyra perforata* (NP); *Porphyra leucosticta* (NA) and *Porphyra fucicola* (NP); *Porphyra* 'amplissima' (NA) and *Porphyra cuneiformis* (NP); *Porphyra* 'umbilicalis' (NA) and *Porphyra mumfordii* (NP). A cluster of NP species including *Porphyra abbotiae*, *Porphyra kurogii*, *Porphyra pseudolinearis*, and *Porphyra pseudolanceolata* formed a sister clade to *P. fucicola* (NP), *P. leucosticta* (NA) and several cryptic NA taxa. These results demonstrate that a number of "independent" migrations occurred between the North Pacific and Northern Atlantic. Furthermore these results confirm the hypothesis, based on similarities in morphology, isozymes, and karyotype, of Lindstrom & Cole (1992), that several sibling species pairs exist for *Porphyra* of the boreal North Pacific (NP) and North Atlantic (NA).

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#### LIGHT REGULATION OF PHYCOBILISOME BIOSYNTHESIS AND CONTROL BY A PHYTOCHROME-LIKE PHOTORECEPTOR

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Ambient light quality changes dramatically affect the composition of light harvesting structures, the phycobilisomes, in many cyanobacterial species. In the cyanobacterium *Fremyella diplosiphon*, shifts in the ratio of red to green light lead to transcriptional changes and altered synthesis of several phycobilisome components. This process is called complementary chromatic adaptation (CCA). These two colors have opposite effects: red light activates an operon encoding the biliprotein phycocyanin (PC) and inactivates the operon encoding phycoerythrin (PE), whereas green light activates PE synthesis and shuts down PC synthesis. The effects of red and green light on CCA are photoreversible. Thus, CCA is similar to transcriptional processes that are controlled by phytochromes, a family of eukaryotic red/far red photoreversible photoreceptors. We are using molecular genetics to determine the mechanisms by which *F. diplosiphon* senses changes in the color of light of its environment. Initial mutant generation and complementation lead to the discovery of three CCA regulatory components that are part of a complex two component system. The most interesting of these is RcaE (regulator of chromatic adaptation), a histidine kinase-class protein containing a region in its amino-terminal half with similarity to the chromophore binding domains of phytochromes. Within this region, RcaE contains a cysteine residue in a similar location as that used for covalent attachment of the open-chain tetrapyrrole chromophore in phytochromes. We will present recent data characterizing RcaE, including in vivo analysis of the chromophore that is attached to RcaE, as well as results from our recent isolation of a new CCA regulatory component.

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### WHAT DOES COMBINING MOLECULAR DATA SETS TELL US ABOUT DIATOM ORIGINS AND PHYLOGENY?

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The past several years have seen an abundance of molecular sequence data gathered on heterokont algae and other stramenopiles with the goal of resolving phylogenetic relationships among major groups. The original focus was on SSU rDNA sequence, but lately a significant number of sequences of plastid and mitochondrial encoded genes (specifically *rbcL* and *coxI*) have been made available. Of particular interest to us has been the origin of diatoms and the relationship of

diatoms to other stramenopiles. According to most claims based on morphological data, typically viewed from a non-rigorous evolutionary taxonomy standpoint (i.e. not with explicit cladistic or phylogenetic systematic methodology), diatoms are closely related to silica-scaled golden brown algae (chrysophytes or synurophytes). SSU rDNA sequence data, however, often place diatoms at the base of the heterokont alga tree, and chryso/synurophytes at the tip with eustigmatophytes, for example, as the chryso/synurophyte sister group. More recent analysis of *rbcL* sequences, however, supports the traditional classification. It is not automatically to be assumed that there is incongruence between the sequences, however. Taxon sampling is different in the different analyses, methods of analysis are often different, assumptions used to "filter" data are different, etc. Moreover, the relative strength of signal appears to be different in the data sets. We will present an analysis of combined SSU, *rbcL* and *coxI* data, an analysis of taxon-sampling issues, and review underlying assumptions and methodologies in an attempt to a) better understand the results of prior studies and b) reconcile the different hypotheses.

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### PHYLOGENETIC RELATIONSHIPS OF GELIDIALES SPECIES IN COSTA RICA

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Despite a general paucity of studies on marine algae in Costa Rica, within the red algal order Gelidiales, 9 species are reported from the Caribbean, and 6 species from the Pacific. In this study 5 species from the southeast Caribbean coast and 2 species from the northwest Pacific coast of Costa Rica were collected for DNA sequence and morphological analyses. At the time of this writing, morphological observations of four Caribbean taxa have identified them as *Gelidium microdenticum*, *Pterocladia capillacea*, *P. melanoidea*, and *Gelidiella acerosa*. In *rbcL* sequence analyses, *Gelidium microdenticum* is resolved within a clade of small *Gelidium* species predominantly found in the south Atlantic. Two collections representing different forms of *Pterocladia melanoidea* sensu Dawson were found to have identical *rbcL* sequences. These samples were resolved in *rbcL* trees with a taxon from Texas identified as *P. bartlettii* and not *P. melanoidea* from Europe. Comparisons with type material are being made to verify these identifications so that the status of *P. melanoidea* in the Caribbean may be determined. The unidentified Caribbean taxon is resolved as a distinct species within the *Pterocladia* clade as well. *Gelidiella acerosa* from Caribbean Costa Rica is closely allied with other Caribbean *G. acerosa* samples in *rbcL* trees. The two pa-

cific taxa are small intertidal turf species and have not yet been identified. Sequence analyses resolve these taxa within a large clade of Indo-Pacific and Caribbean *Gelidium* species.

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### DIATOMS AS HOSTS FOR OTHER DIATOMS: OBSERVATIONS FROM SOUTHERN CALIFORNIA

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Large epiphytic and epilithic diatom species hosting other diatoms were observed in several fresh- and brackish water sites in Southern California. The most commonly encountered hosts were species forming long filaments attached to rocks or macroalgae, *Hydrosera whampoensis* (Schwarz) Hendey, *Melosira varians* Agardh, *Pleurosira laevis* (Ehrenberg) Compere and *Terpsinoe musica* Ehrenberg. These large diatoms often had smaller diatoms attached, usually to the girdle bands and occasionally to the mucilage pads connecting the cells. For example, cells of *T. musica* were observed supporting growth of a diverse diatom assemblage composed of species of the genera *Achnanthes*, *Achnantheidium*, *Amphora*, *Cocconeis* and *Tabularia*; *Synedra* sp. was attached to *M. varians* and *B. paxillifer*; and *Cocconeis placentula* was seen on *H. whampoensis*. Thus, large epiphytic and epilithic diatoms seem to provide suitable sites for attachment of small epiphytic diatom species, and it appears that this phenomenon is more common than previously thought.

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### ASSOCIATION OF A FREE-LIVING EUGLENA WITH A COLONIAL ROTIFER

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*Euglena agilis* (syn. *Euglena pisciformis*) is a free-living euglenoid found in brackish or fresh water. This species can be identified by its fusiform shape, single long flagellum, and prominent anterior eyespot. It can be distinguished from other euglenoids in part by its two to three large chloroplasts that run parallel to the length of the body, each with a single pyrenoid capped on two sides by paramylon. *Euglena agilis* is commonly found in our collecting ponds in central New Jersey. In two consecutive summers, we have found large numbers of these cells living symbiotically

with the colonial rotifer *Conochilus unicornis*. We do not know if either organism benefits or is harmed in this relationship. For example, while we've seen the rotifers of the colony ingest other algae, we have not seen predation of the *E. agilis* (neither those embedded in the mucilaginous secretions of the rotifer nor swimming outside of the colony). This association is interesting for several reasons: 1) *E. agilis* is normally free-living. 2) There are few known symbiotic relationships between euglenoids and rotifers. 3) *E. agilis* lose their long single flagellum when in the colony. We plan to use light and electron microscopy to investigate the ultrastructure of the *E. agilis* and its relationship to the rotifer colony.

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### EVIDENCE FOR GLUCAN COMPONENTS IN THE PRIMARY ZYGOTE WALL OF *CHLAMYDOMONAS MONOICA*

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The primary zygote wall of *C. monoica* is transient and is released from mature zygospores. The fluorochromes aniline blue and primulin, used in other systems to detect  $\beta$ -1,3 glucans, stain the primary wall intensely. Two  $\beta$ -1,3 glucan synthases have been identified in higher plants: a calcium-dependent synthase produced in response to wounding and induced by chitosan, and a magnesium-dependent enzyme, associated with pollen development and unresponsive to chitosan. Chitosan has no effect on *C. monoica* primary wall synthesis or staining properties. We are presently testing for the effect of magnesium and/or calcium depletion on primary wall synthesis. Aniline blue and primulin do not stain purified cellulose fibers, while the fluorochrome Calcofluor does. Calcofluor also stains the primary wall intensely. For all fluorochromes tested, fluorescence is first detected in motile quadriflagellate zygotes. Aniline blue staining maximizes quickly, while Calcofluor staining continues to intensify until primary wall release. Dinitrobenzotrile, a specific inhibitor of cellulose synthesis in plants, has no effect on primary wall synthesis in *C. monoica*. Addition of glucanase or cellulase to partially purified primary walls results in wall thinning and loss of staining. Using electron microscopy, we are evaluating the effects of these enzymes on primary wall ultrastructure. Further studies are needed to determine whether all three fluorochromes are recognizing the same polysaccharide component (a  $\beta$ -1,3 glucan or a  $\beta$ -1,3;  $\beta$ -1,4 mixed glucan), or whether Calcofluor staining indicates the presence of a distinct component containing  $\beta$ -1,4 linkages, such as cellulose or a xyloglucan.

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### **SURVEY OF BENTHIC DIATOM COMMUNITIES FROM LOTIC SYSTEMS WITHIN THE WESTERN ALLEGHENY PLATEAU**

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Over the course of 3 years (1997–1999), 72 stream sites were sampled for epilithic diatom communities. The analysis of these samples has led to the identification of over 325 species of diatoms. In addition to sampling the diatom community, selected physical and chemical parameters were recorded from each stream reach. These parameters included pH, specific conductance, current velocity, SRP, nitrate, silica, and total alkalinity. Canonical Correspondence Analysis (CCA) was used to identify influential environmental parameters and to assess the response of the diatom community to prominent anthropogenic inputs in the region (i.e. coal mine drainage, eutrophication). The initial analyses indicate that pH was the most influential environmental parameter along the first CCA axis. This shift was not unexpected, as acid mine drainage (AMD) in the region leads to a wide range of pH values (2.8–7.93). The highly acidic sites were characterized by species of the genus *Eunotia* (specifically *E. exigua* and *E. steineckei*), *Frustulia rhomboides*, and *Pinnularia subcapitata*. Furthermore, *Achnanthydium minutissimum* was the most widely distributed of the diatom species encountered, being found at 94% of the sites sampled. Streams that fluctuated between acidic and circumneutral pH (termed “teeter-totter”) had greater abundances of *Brachysira vitrea* than other streams in this survey. Further implications for the use of these diatom communities as biomonitoring tools and the distribution of assemblages within the Western Allegheny Plateau will be discussed.

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### **BIOGEOGRAPHY OF *BATRACHOSPERMUM HELMENTOSUM* (BATRACHOSPERMALES, RHODOPHYTA) IN NORTH AMERICA USING MOLECULAR AND MORPHOLOGICAL DATA**

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Twelve collections of the freshwater red alga *Batrachospermum helmentosum* were investigated using molecular sequence and morphological data. Collections were from streams throughout the range of this species in North America, from Louisiana and Tennessee in the south to Michigan and Massachusetts in the north. Eleven morphometric characters were measured for each of the specimens sequenced. Both the

rubisco large subunit gene (*rbcl*) and the ribosomal DNA internal transcribed spacers (ITS1 & 2) were sequenced. Morphometric results showed individuals from the various locations to be morphologically uniform with little variation in pertinent characters. In the analysis of the *rbcl* data, samples from Connecticut, Ohio, Louisiana, North Carolina and Indiana were identical in sequence and the Rhode Island and Massachusetts samples were most derived. The Michigan and Tennessee samples were closely related to each other but not the other samples. For the ITS data, the tree topology was similar to that obtained with the *rbcl* data. In comparison with *rbcl*, sequence variation in the ITS region among samples was greater and showed more resolution among the stream sites. In terms of biogeography, three geographically close samples (Connecticut, Rhode Island, Massachusetts) were most closely related. However, genetic relationships did not necessarily reflect geographic distance. For example, two distant populations, Louisiana and Indiana, were most closely related. Variation in ITS sequence data among individuals from a single stream site will be discussed.

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### **THE PHYLOGENETICS OF ACROCHAETIALES REVISITED: INFERENCES FROM *RBCL* AND CHLOROPLAST ENCODED 23S *RRNA* SEQUENCE ANALYSIS**

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Recent molecular evidence from 23S rRNA sequences has shed light on the phylogenetic status of Acrochaetiales and Palmariales, suggesting a paraphyletic Acrochaetiales and a monophyletic Palmariales-Acrochaetiales assemblage characterized by the absence of both the carpogonial filament and associated post-fertilization fusions. It has also brought into question the status of Palmariales. Despite these findings, the familial and generic systematics of Acrochaetiales have remained somewhat obscure, in large part due to inadequate taxon and gene sampling. In the present study we address this problem by performing phylogenetic analyses of *rbcl* and 23S rRNA sequences from acrochaetioid algae covering a broad range of morphologies. Our results confirm elements from several primarily chloroplast based classification schemes (Papenfuss 1945, 1947; Stegenga 1979, 1985; Lee & Lee 1988) while also providing new phylogenetic insight. Two groups are supported at the most inclusive level. Group I is marine, has parietal chloroplasts, and includes three clades delimited primarily by the presence/absence of pyrenoids, number of chloroplasts,

and possibly spore germination pattern. Group II is more heterogeneous and consists of three clades differentiated largely by chloroplast type (stellate/parietal, pyrenoid present/absent) and number, habitat (marine/freshwater) and life history. Systematic criteria drawn from the closely related order Palmariales suggest that we recognize Group I as a new family, Colaconemataceae, and the three taxa in Group II as Acrochaetiaceae, Rhodochortonaceae and Audouinellaceae. Furthermore, each of the three groups in Colaconemataceae could be regarded at the generic level. We also report on the utility of 23S rRNA for red algal systematics.

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### THE ROLE OF CALCIUM IN FLOW-STIMULATED BIOLUMINESCENCE OF THE RED TIDE DINOFLAGELLATE *LINGULODINIUM POLYEDRUM*

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Many marine planktonic dinoflagellates emit flashes of light in response to either laminar or turbulent flows as well as direct mechanical stimulation. The production of a flash of light is known to be mediated by a proton-mediated action potential across the vacuolar membrane; the mechanotransduction process initiating this action potential is unknown. Here we report on an investigation into the role of  $Ca^{+2}$  in the mechanotransduction process regulating bioluminescence in the red tide dinoflagellate *Lingulodinium polyedrum*. Calcium ionophores and low concentrations of the membrane-disrupting agent digitonin stimulated bioluminescence only when calcium was present in the media or added with the agent, indicating that the flash-triggering vacuolar action potential is specifically stimulated by a calcium influx. A variety of known calcium channel blockers or antagonists inhibited mechanically stimulated bioluminescence but did not affect cellular bioluminescent capacity. In many cases the inhibitory affect occurred after only a brief exposure. In addition, gadolinium ( $Gd^{+3}$ ), a blocker of many stretch-activated ion channels, caused potent inhibition of mechanically stimulated bioluminescence. The order of potency of the transition metals tested was  $La^{+3} > Gd^{+3} > Co^{+2} > Mn^{+2} > Ni^{+2}$ , similar to their potency as blockers of known calcium channels. Experiments with a quantified shear flow demonstrated that flow-stimulated bioluminescence depended on the level of extracellular calcium. Future work will elucidate the signaling pathway involving calcium-mediated flow-stimulated mechanotransduction. Our goal is to use bioluminescence as a proxy for the initial cellular mechanotransduction events triggered by fluid flow.

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### TEMPERATURE INDUCED PHOTOINHIBITION IN OUTDOOR CULTURES OF *MONODUS SUBTERRANEUS*

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Outdoor algal cultures are continuously exposed to changes in environmental conditions, particularly irradiance and temperature. While the changes in light intensity take place in a range of one to two hours, the increase in temperature is a slower process and takes about four to five hours. This de-synchronization between the two important environmental factors governing photosynthesis and growth of algae results in a unique stress condition where photoinhibition can be induced at relatively low light intensity. Outdoors the early morning culture temperature was found to be about 12 to 14° C, and reaches 25 to 28° C at mid-day. In an experiment, such a natural temperature regime was compared to another one in which the morning temperature of the culture was increased to 20° C by using a heating system. A fast decline in the maximal photochemical efficiency of  $PS_{II}$  ( $F_v/F_m$ ) was observed starting as soon as sunrise. The decline was faster in the non-heated culture and was to a lower value. The diurnal changes in the electron transfer rate (ETR) and in the non-photochemical quenching (NPQ) of the cultures, indicated that the early morning exposure of cells to sub-optimal temperature results in a fast inactivation of  $PS_{II}$  activity which was reflected in an inhibition of the photosynthetic activity even when the two cultures finally reached the same temperature at mid-day. Thus, under the same light and temperature mid-day conditions the ETR was higher and the NPQ was significantly lower in the heated culture. Significant changes in productivity of the cultures also were observed.

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### *FUCUS* VANADIUM PEROXIDASE: MINIMUM CATALYTIC DOMAIN SIZE RETAINING PEROXIDASE ACTIVITY

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Vanadium peroxidase catalyzes the extracellular assembly of *Fucus* zygote cell surface adhesive (Vreeland & Epstein 1996, *Modern Meth. Plant Anal.* 17, 95–116). Our goal is to identify the catalytic, self-associating

and wall targeting functional domains of algal vanadium peroxidase to understand its role in algal propagule adhesion. As a first step, we truncated our recombinant *Fucus* vanadium peroxidase (GenBank AF053411) for catalytic domain identification. Recombinant constructs were prepared which reduced the C-terminal catalytic domain at either or both N- and C-terminal ends. Recombinant proteins were expressed in *E. coli*, refolded from cytoplasm and inclusion bodies and tested for vanadium-specific oxidase activity. Preliminary results demonstrated peroxidase activity when the 40 kDa catalytic domain was truncated on both ends to 24 kDa. Further terminal and internal truncation is needed to fully define the minimal catalytic unit, which could be as small as 15–20 kDa within the 73 kDa monomer. The very small catalytic unit in *Fucus* vanadium peroxidase is not unexpected considering the rigid undulated helical vanadate frame in the *Curvularia* fungal vanadium peroxidase (Macedo-Ribeiro *et al.* 1999, *J. Biol. Inorg. Chem.* 4, 209–219). We conclude that interactions between the N-terminal non-catalytic domain and the C-terminal catalytic domain, found in the crystalline *Ascophyllum* enzyme (Weyland *et al.* 1999, *J. Mol. Biol.* 293, 595–611), are unnecessary for peroxidase activity. Other conserved amino acids in the C-terminal half of *Fucus* vanadium peroxidase, peripheral to the helical core, could participate in protein surface functions such as self-association and wall targeting.

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#### DIATOM SILICA BIOMINERALIZATION: AT NANOSCALE LEVEL A CHEMICALLY UNIFORM PROCESS

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Using a high-brilliance synchrotron X-ray source, combined small- and wide-angle X-ray scattering (SAXS and WAXS) was applied to study nanoscale characteristics, in particular pore size in the range of 3 to 65 nm, of a variety of unialgal cultures of centric and pennate diatoms, and of mixed diatom populations sampled in the field. Results of scattering analysis were compared with details of pore size, structure and orientation visible at the electron microscopic level. WAXS patterns did not reveal any crystalline phase or features of microcrystallinity (resolution 0.07 to 0.51 nm), which implies a totally amorphous character of the SiO<sub>2</sub> matrix of the frustule material. SAXS data (resolution 3 to 65 nm) provided information on geometry, size, and distribution of pores in the silica.

Overall, two pore regions were recognized that were common to the silica of all samples: the smallest (d less than 10 nm) regularly spaced and shaped spherically, the larger (up to 65 nm) being cylinders or slits. Apparently, at a nanoscale level diatomaceous silica is quite homologous among species, in agreement with the chemical principles of silica polymerization under the conditions of pH and precursor concentrations inside the silicon deposition vesicle. The final frustule “macro”-morphology is of course species-specific, being determined genetically. Synthetically-derived MCM-type silicas have a similarly organized pore distribution in an amorphous silica matrix as we found in all diatom species studied. We therefore suggest that organic molecules of a kind used as structure-directing agents to produce these artificial silicas play a role in the nucleation of the silica polymerization reaction and the shaping of pore morphology inside the silicon deposition vesicle of diatoms. Structure-directing molecules now await isolation from the SDV, followed by identification and characterisation by molecular techniques.

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#### A REASSESSMENT OF ITS/5.8S DNA SEQUENCE VARIATION AND PHYLOGENETIC SIGNAL IN THE SYNUROPHYCEAE

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Previously, DNA sequences were determined for the entire ITS/5.8S region by direct sequencing of PCR amplification products. 28 sequences were obtained from 12 species of Synurophyceae, including *Tessellaria volvocina*, *Chrysodidymus synuroideus*, six species of *Synura* and four species of *Mallomonas*. Sequences also were determined from multiple strains of some species, including 15 strains of *Synura petersenii*. The length of these sequences varied from 495–598 bp. The large amount of ITS/5.8S length variation made initial attempts in determining reliable sequence alignment difficult and precluded their use in phylogenetic analyses of the class. Subsequently, the approach of Bogler & Simpson (1996) was investigated where reiterative, computer assisted alignments were generated and the quality of the alignment was evaluated by homoplasy index and consistency index values. However, these alignments also were equivocal. ITS/5.8S sequences sorted the 15 *S. petersenii* strains into two well supported ITS lineages. One ITS type included ten isolates from North America while isolates from the second ITS type included one each from Germany and Australia and three from North America. Results are presented here where the sequences from the 15 *S. petersenii* isolates were used to characterize ITS-1 and ITS-2 RNA transcript secondary structure. Next, this information was expanded to include ITS/5.8S sequences from the other taxa in the class.

Finally, the characteristics of ITS 1 and ITS 2 RNA transcript secondary structure were used as a guide to align all of the ITS/5.8S sequences from the Synurophyceae in a final phylogenetic analysis.

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**BIOGEOGRAPHY OF ASEXUAL AND SEXUAL REPRODUCTION IN CALOGLOSSA (DELESERIACEAE, RHODOPHYTA) FROM AUSTRALIA AND NEW ZEALAND**

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*Caloglossa* species are widely distributed in mangroves and salt marshes around the world and their life history patterns are being investigated in laboratory culture. In Australia all isolates of *C. monosticha*, *C. postiae* and *C. ogasawaraensis* have *Polysiphonia*-type (P-type) sexual life histories. Among the 70 *C. lepriurii* isolates from Australia and New Zealand P-type sexual reproduction also is dominant. However, ten isolates of *C. lepriurii* from the Spencer Gulf and the Gulf of St. Vincent in South Australia give rise to successive tetrasporophyte generations without gametophytes. Moreover, one isolate from Queensland is asexual. Only one South Australia isolate, obtained from Lake Alexandrina at the mouth of the Murray River, is sexual. South Australia and Pacific Mexico are two regions in which asexual reproduction is dominant. In another mangrove dwelling red alga *Bostrychia moritziana* (Rhodomelaceae) non-sexual reproduction also is frequent in Australia, New Caledonia and Bali (Indonesia). This asexual reproductive pattern of tetrasporophytic recycling appears to have arisen independently among individual populations of various red algal species in different regions. Investigations are underway on the molecular phylogeny of the *Caloglossa lepriurii* isolates.

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**ANABAENA AND MICROCYSTIS IN THE PACIFIC NORTHWEST: A TALE OF PLASTICITY**

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The molecular marker, cpcBA-IGS, was used to examine the biogeography of representatives of *Anabaena* and *Microcystis* collected from Pacific Northwest lakes and their phylogenetic relationship to isolates from the UTEX and CCAP culture collections. cpcBA-IGS contains portions of the alpha and beta subunit of cyanophycocyanin with an intergenic spacer (IGS) of ~100 base pairs situated between the subunits. The

use of this cyanobacteria specific marker and primers allowed us to amplify cpcBA-IGS DNA from uni-cyanobacterial field samples. The morphology and habit of specimens were documented by photomicroscopy. Single filaments or colonies of field material and 3–4 filaments of culture material were then amplified using the polymerase chain reaction (PCR). Results include analysis of non-toxic summer and toxic winter blooms of *Anabaena* from 1989–1997 in American Lake, WA and a comparison of non-toxic and toxic *Microcystis* from Lake Sammamish, WA. A preliminary phylogenetics analysis of Pacific Northwest and UTEX isolates also was performed using parsimony, maximum likelihood and distance methods. The results of this analysis suggest that revision of *Anabaena* and *Microcystis* taxonomy may be needed.

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**MECHANISMS CONTROLLING NUCLEAR MIGRATION ALONG THE TRICHOGYNE IN THE RED ALGA, BOSTRYCHIA MORITZIANA (RHODOMELACEAE, RHODOPHYTA)**

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Sexual reproduction in red algae has been studied for over a century primarily for taxonomic purposes. Despite this interest, only recently have the critical events of fertilisation been revealed. Time-lapse video microscopy techniques have been used to document the stages in the fertilization process within *Bostrychia moritziana*. Once plasmogamy between a trichogyne and spermatium is achieved, two differentiated male nuclei may enter the trichogyne. One of the two migrates to the carpogonium while the other travels in the opposite direction towards the trichogyne tip and plays no further role in fertilisation. The precise mechanism governing this bidirectional movement of male nuclei is unknown, but the cytoskeleton is believed to be involved. Fluorescent staining and confocal microscopy shows an extensive arrangement of actin filaments in spermatia and along trichogynes yet microtubules are only evident in dividing spermatia. Actin filaments appear to be involved in plasmogamy and ensheath male nuclei as they migrate from the spermatium and as they travel along the trichogyne in either direction. Drug-inhibition studies and UV-microbeam irradiation indicate nuclear migration along trichogynes is actin/myosin dependent

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**MITOSIS, CELL WALL AND FLAGELLA SYNTHESIS IN SYNCHRONIZED CULTURES OF THE PRASINOPHYTE, SCHERFFELIA DUBIA**

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Cell division occurs within the parental cell wall, yielding two progeny cells. Since *Scherffelia dubia* sheds all four flagella prior to cell division, the maturing progeny cells must regenerate new cell walls and flagella during and/or after cytokinesis. To better understand these processes, we have synchronized cell division in cultures of *S. dubia* and observed all stages of mitosis, cytokinesis, and progeny cell maturation, including flagella and cell wall formation, via DAPI staining of fixed cells, DIC microscopy of live cells embedded in agarose and standard TEM. Microscopical observations revealed the following sequence of events: 1) Golgi stacks divide during late interphase and immediately begin producing theca scales; 2) deflagellation and release of the parental cell wall from the plasma membrane occurs during early prophase; 3) synthesis of theca and flagella scales within the Golgi and/or scale reticulum continues throughout mitosis; 4) during cytokinesis, a coalescence of vesicles containing theca scales at the posterior end of the cell results in a cleavage furrow slightly diagonal to the cells' longitudinal axis (40 min); 5) post-mitotic nascent basal body formation and flagella elongation at the inherited basal bodies (and later at the mature nascent basal bodies) occurs concurrently with continued cell wall synthesis; 6) the cleavage furrow rotates into a transverse position (35 min); 7) reorientation of the nuclei results in a "head to tail" orientation of the maturing progeny cells; and 8) matured progeny cells emerge from the posterior end of the parental theca not before 8 hrs after the onset of mitosis.

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#### MARINE MACROALGAL DIVERSITY IN THE REPUBLIC OF PANAMA

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The marine flora of Panama remains poorly described to date, having been described from a few sporadic and species poor (<50 species) collections in the early half of the century and a couple of dedicated surveys in the latter half. With the exception of a few studies that have focused on particular genera or species, only a single marine floral survey has been published in the past three decades. In 1999 we collected marine algae from nearly 100 different collection sites along both coasts of Panama over a 10 month period of time. Over 1500 specimens have been curated, representing 250 Caribbean species and 117 Pacific species. This is an increase in algal diversity of approximately 100 and 50% for the Caribbean and Pacific flora, respectively, relative to Earle's compilation of 1972. We estimate that algal diversity in Panama may approach 450 species as the remaining 30% of the col-

lection is identified and previous records are incorporated. Aspects of biogeography will be discussed in light of such extraordinary diversity over a relatively confined region.

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#### IN SITU GROWTH AND REPRODUCTION OF PORPHYRA PERFORATA IN THE PACIFIC COAST OF BAJA CALIFORNIA

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*Porphyra perforata* is a common species off the Pacific coast of Baja California peninsula. *In situ* growth, phenology and reproductive characteristic of this plant are being determined in order to evaluate the feasibility of its culture in open water conditions. Contrary to previous studies that describe this species as ephemeral, *P. perforata* is present all year around off the north coast of the Baja California peninsula. Its vertical distribution is limited to a band, +50 to +150 cm above sea level. However, subtidal growth transplant experiments showed that the plants are capable of growing down to a depth of 5 m. Highest growth rates were observed from February to July, then tissue was lost as a consequence of sexual reproduction. Biomass continues to decrease for the rest of the year. About 50% of the plants were reproductive by May and more than 90% by August. Oysters shells were introduced and collected every month to evaluate the presence of chonchocelis phase and *P. perforata* recruitment. Chonchocelis was observed all year and new recruits were present from September to February. These results suggest that the foliar phase *P. perforata* could be cultured in the ocean for about six months of the year and could be culture subtidally.

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#### CONFIRMATION OF CATFISH MORTALITIES RESULTING FROM MICROCYSTIN PRODUCED DURING MICROCYSTIS BLOOMS

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Enigmatic deaths of finfish have occurred in freshwater systems for many years. Some of these fish mortali-



ties can be attributed to the accumulation of cyanobacterial toxins during ingestion of floating feeds or passively through opercular ventilation. We recently were able to document the accumulation of microcystin and resulting catastrophic mortality of pond-raised catfish (*Ictalurus punctatus*). The causative organism, *Microcystis aeruginosa*, was the dominant phytoplankton component within the cooler fall-winter season. Microcystin, a hepatotoxin produced by *Microcystis*, was detected in water and catfish liver samples. Fish exposed to pond water containing this bloom were killed within 24 h. Necropsy of fish exposed to pond water revealed congested liver and spleen tissues. Environmental conditions associated with several similar bloom events suggest several predictive options to forecast conditions supporting toxin formation, particularly temperature.

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#### MICROSCOPIC INVESTIGATIONS OF *STROMBOMONAS* WITH SPECIAL REFERENCE TO RELEASE FROM THE LORICA FOLLOWING CELL DIVISION

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The Euglenophyta include four genera of *Euglena*-like cells enclosed in a lorica. Two of these genera, *Trachelomonas* and *Strombomonas*, are free swimming. These taxa have been differentiated and classified at the generic and species level based primarily on the morphology of their lorica. Numerous SEM studies have been conducted, yet few, if any, TEM descriptions exist, especially for *Strombomonas*. This study describes both the outer lorica and internal ultrastructure for *Strombomonas* sp. isolated from a fresh water pond in New Jersey. Additionally, we describe the release of daughter cells from the lorica following cell division. Two cells were observed within a single *Strombomonas* lorica following cytokinesis. When exposed to the light of the microscope, one or both of the cells

emerged through the neck of the lorica. The released cells could easily be mistaken for *Euglena* species.

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#### FIVE CRYPTIC SPECIES OF *PORPHYRA* FROM THE NORTHWEST ATLANTIC

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As one part of a multi-institutional project on the biology of *Porphyra*, we collected extensive population samples of native *Porphyra* species from Long Island Sound to Nova Scotia. Samples were initially identified to species based on morphological features using taxonomic keys in Bird & McLaughlin (1992). Collections were analyzed using starch gel electrophoresis to look for intra-specific variation useful in assessing population genetics. Isozyme variation in samples that had been originally identified as *Porphyra leucosticta* was far greater than expected. Samples from some collections differed by as much as 7 out of 7 loci. Other investigators have used starch gel electrophoresis to reveal cryptic differences in *Porphyra* that led to the descriptions of new species. In light of our isozyme results, we began a thorough examination of what had been identified as *P. leucosticta* in the northwestern Atlantic. The study included new collections as well as herbarium specimens. We examined morphology, habitat, seasonality, reproductive patterns, cell size & shape. We also extracted DNA and sequenced 1484bp of the *rbdL* gene. Based on this evidence, we feel we can circumscribe at least five distinct taxa of *Porphyra* not previously described from the northwestern Atlantic. Whether these represent new species, or previously described taxa from other geographic regions, we are not yet certain. We present here in detail, descriptions of each species along with summarized results of isozyme and DNA analyses.