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RELATIONSHIP OF WALL BIOCHEMISTRY TO ENVIRONMENTALLY INDUCED MORPHOLOGICAL CHANGES IN THE DIATOM, *PHAEODACTYLUM TRICORNUTUM* (BACILLARIOPHYCEAE).

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An important adaptation of diatoms to the environment is the production of extracellular polymeric substances (EPS) that are mainly composed of sulfated polysaccharides. We used the fast growing pennate polymorphic diatom *Phaeodactylum tricornutum* Bohlin as a model to understand how morphological changes and wall biochemistry are affected by environmental conditions. P limitation resulted in reduced growth rate but did not affect the morphology of *P. tricornutum* as both P replete and P limited cultures were dominated by the fusiform morphotype. While cultures in salinity levels lower than 45 ppt were enriched in the fusiform morphotype, cultures in higher salinities (> 45 ppt) or in solid media resulted in more oval morphotypes. Carbohydrate production increased about 2 fold when cultures were P limited, but only slightly increased with increasing salinity levels. The monosaccharide composition of the polymers varied with the P status of the media, salinity levels, and whether cultures were in liquid or solid media. Eight neutral sugars were detected, with the levels of glc, rha, man, gal, and xyl being the most affected by changing environmental conditions. As evidenced by changes in monosaccharide profiles, sulfate content, and toluidine blue staining, the sulfated glucomannans closely associated with *P. tricornutum* wall are altered coincident with environmentally induced morphological changes. We are currently exploring the regulation of putative mannosyl transferases and sulfotransferases as related to environmental alterations in wall biochemistry with morphotype changes coordinated.

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APPLICATIONS OF THE LISST-100 TO THE STUDY OF PHYTOPLANKTON ECOLOGY

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The abundance and size distribution of phytoplankton largely affect biochemical processes and food web dynamics in the ocean. While microscopy provides an accurate measurement of cell size and concentration this method is time consuming and tedious. This paper examines the use of an optical particle sizing technique to obtain rapid measurements of phytoplankton size distribution and volume concentration. Experiments using the LISST-100 (Laser In Situ Scattering and Transmissometry) instrument, designed by Sequoia Scientific, were performed to obtain qualitative and quantitative information about six different species of marine phytoplankton cultured in the laboratory. Beads of known size and optical properties were used to calibrate and test the sensor. The LISST's results were compared to traditional microscopy measurements. For phytoplankton cells with a near spherical shape the LISST provided a robust estimate of the size distribution. For more complex shapes of phytoplankton (e.g. due to spines, shape and chains), the LISST provided a broad size range. Additionally, the LISST provides a good estimate of the total cross sectional area per volume of the cells, and therefore a good estimate of cell concentration. Our results suggest that the LISST is a rapid and accurate method to measure phytoplankton cells size and concentration in laboratory cultures and is likely to be useful for phytoplankton ecology studies in the field.

DEVELOPMENT OF A METHODOLOGY TO MEASURE IN SITU CELL DIVISION RATE (CDR) FOR *EMILIANIA HUXLEYI*.

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Understanding bloom dynamics of *Emiliania huxleyi* (Ehux), a coccolithophorid phytoplankton, is of pivotal importance for better characterizing its role in oceanic carbon flux and dimethylsulfide (DMS) production. Toward this goal, we are developing a proliferating cell nuclear antigen (PCNA)-based molecular technique to estimate the cell division rate (CDR) of Ehux populations. This process requires sequencing of the Ehux PCNA gene and development of probes, such as antibodies or PCR primers. The generated antibodies can then be used to detect the proportion of cells expressing PCNA (i.e. in the S phase of the cell cycle) by light microscopy. PCR primers, on the other hand, can be used in Real-Time quantitative RT-PCR to quantify the amount of PCNA mRNA in the cell population. We hope that both approaches will converge in the estimation of CDR in Ehux populations. So far, we have sequenced PCNA from Ehux and are expressing its encoded protein in E. coli for antibody production. Meanwhile, we have designed specific primers for Real-Time PCR. Some results of Ehux PCNA expression pattern will also be presented.

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A PHYLOGENETIC AND GENOMIC PERSPECTIVE ON THE ORIGINS OF PHOTOSYNTHETIC EUKARYOTES WITHIN THE TREE OF LIFE.

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The algae and plants are distributed among many, often distantly related branches of the tree of life. Determining the evolutionary relationship of these photosynthetic eukaryotes and how their photosynthetic organelle (plastid) has arisen are central problems in evolution. Recent molecular phylogenetic and genomic data suggest strongly that all plastids ultimately trace their origin to an ancient (ca. 1.5 billion years ago) primary endosymbiotic event in which a previously non-photosynthetic protist engulfed and enslaved a cyanobacterium. This first photosynthetic eukaryote gave rise to the red, green, and glaucophyte algae (often called the Plantae). However, other lineages, such as the chlorophyll *c*-containing algae have a more complicated evolutionary history involving a secondary endosymbiotic event, in which a protist engulfed an existing eukaryotic alga. Following this eukaryotic engulfment(s), the chlorophyll *c* algae such as diatoms, kelps, and dinoflagellates rose to great importance in aquatic habitats. In this talk, data will be presented that provide a foundation for understanding the origin and diversification of photosynthetic eukaryotes. Phylogenetic analyses will be used to trace the origins of the Plantae, the chlorophyll *c*-containing algae (often called the chromalveolates), and their plastids. In addition, a phylogenomic approach will be used to assess the impact of endosymbiosis on nuclear genome evolution. In this method, phylogenetic inventories are made for each conserved (i.e., "annotatable") gene in a genome to understand its origin, for example, through endosymbiotic gene transfer, and its evolution. These data identify algae as some of the most interesting taxa on our planet that can provide fundamental and general insights into the course of eukaryotic evolution.

SEASONALITY AND ENVIRONMENTAL EFFECTS ON *PORPHYRA UMBLICALIS* FROM MAINE, U.S.A.

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Porphyra umbilicalis is an aseasonal intertidal red alga that is common throughout the North Atlantic Ocean. In the western Atlantic, the gametophytic phase is reported to be capable of reproducing both asexually and sexually. We are collecting material from Blueberry Hill on Schoodic Peninsula, Maine each month to determine when *P. umbilicalis* reproduces sexually and asexually. No sexually-reproducing thalli were found from May (2004) – March (2005). The effects of water motion, irradiance, temperature, and photoperiod on aplanospore production were investigated. Aplanospores were formed and released under each set of variables, with the rate of reproductive maturity slower in 5^{o} versus 10^{o} and 15^{o} Celsius treatments. Results also show that still water is inhibitory to reproductive maturation. When aplanospores were produced in calm treatments, they were not released from the tissue disk and germinated *in situ*. We are currently investigating the effects of bacterial association on aplanospore production in the laboratory. We plan to examine the effect of desiccation on growth and reproductive maturity in *P. umbilicalis* at the University of Maine's Center for Cooperative Aquaculture Research (CCAR) beginning this spring. This summer, we will grow out conchospore-generated *P. amplissima* and aplanospore-generated net cultures of *P. umbilicalis* alongside salmon pens in Cobscook Bay to investigate the feasibility of using these species in integrated aquaculture in an open water setting. (Supported by an EPA STAR Fellowship, a Maine Sea Grant, and a Maine Technology Institute Seed Grant.)

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THE PARADOX OF THE PLANKTON AND OTHER STORIES: USING A NEW MODEL TO ADDRESS OLD QUESTIONS.

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The mechanisms regulating taxonomic composition of biological systems are poorly understood. However, the degree to which organisms within an assemblage are specialized to exploit specific portions of a resource gradient (i.e. "niche breadth") may provide some insight into the importance of some general factors. As diatom niche is determined largely by habitat, diatom assemblages were used as a simple model system. A non-linear decay model was used to evaluate the habitat specificity of different levels of organizational hierarchy within diatom assemblages of various systems. Values generated by the model were then compared to a suite of geographical and environmental factors. Although this work is still preliminary, it has generated some interesting results relating to latitude, disturbance, and endemism.

TASTE PREFERENCE AND FATTY ACID CONTENT OF *PORPHYRA* SPP.

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We are participating in a regional project with other American and Canadian phycologists to develop native species of *Porphyra* as suitable crops for polyculture. To determine which species to select, we are developing nutritional information on native species (e.g., omega-3-fatty acid content) and elucidating American consumers' taste preferences. We selected two native species for tests based on their local abundance and anecdotal impressions of taste. Our results suggest that the American palate will accept native species of *Porphyra* in food products. Taste tests between *P. umbilicalis* and *P. amplissima* and between *P. amplissima* and *P. yezoensis* were conducted with both children and adults. Although the level of acceptance was different in tests with adults than with children (2^{nd} and 5^{th} graders), there were no significant differences between species in either test ($p > 0.50$) for either test group. These results suggest that the American palate will accept native American species of *Porphyra* in food products. Lipids were extracted from the material used in taste tests with a modified Folch procedure, and GC/MS analysis used to determine the fatty acid profile. Palmitic and eicosapentaenoic (EPA) acids were the most abundant fatty acids in both species; EPA, an omega-3-fatty acid, was present at 0.9 mg/g dry wt in *P. amplissima* and 0.4 mg/g dry wt in *P. umbilicalis*. American consumers' acceptance and preference for different *Porphyra* spp. is important information to select species for large-scale mariculture. (This work is supported by National Science Foundation K-12 grant 9979581 and Maine Sea Grant 0054 NA030AR417.)

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TWO ASIAN SPECIES OF *PORPHYRA* FOUND ON THE NEW ENGLAND COAST.

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Porphyra yezoensis is a red seaweed native to the Asian Pacific where it is grown extensively for food (nori sheets) by coastal net culture. There have been two attempts to farm *Porphyra* in the United States: one in Puget Sound and the other in Cobscook Bay near Eastport, Maine. Concerns over the introduction of non-indigenous species along the New England coast prompted a study funded by Sea Grant Non-indigenous Species Program during the operation of the Cobscook Bay farm in which no permanent populations of *P. yezoensis* were found. However, in the past two years, significant intertidal populations of *P. yezoensis* have been discovered in Maine, New Hampshire, Massachusetts, Connecticut, and New York. Molecular comparisons of the cultivars grown in Cobscook Bay and the recently discovered populations confirm that the aquaculture operation was not the source of these introductions. Based on *rbc*L and ITS1 sequences, the populations north of Cape Cod in Massachusetts match a Japanese cultivar (F6-1) and likely represent expansion from a single introduction. Populations south of the Cape match a second Japanese cultivar (NA-4) and indicate a second introduction. Additionally, in the past year we have discovered a second Asian species, *P. katadae*, at three locations north of Cape Cod and one location south of Cape Cod. *P. katadae* has never been reported in the Atlantic. Kornman (1986) reported finding a population of *P. yezoensis* on Helogland, Germany, which he identified based on morphology and characteristics in culture. Kornman's identification has subsequently been questioned based on molecular evidence.

PROTIST EST DATABASE: AUTOMATED ANNOTATION AND DATA REPRESENTATION.

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PEPdb is a taxonomically broad eukaryotic database that organizes and integrates protist cDNA sequences generated by the pan-Canadian Protist EST program (PEP). The aim of this collaborative project is to characterize the expressed genome portions of ~30 poorly-studied protists, including photosynthetic taxa such as chlorophytes, glaucophytes, dinoflagelates; prototrophic mitochondriate protists such as lobosea, cercomonads, ciliates, euglenozoa, jakobids; and amitochondriate protists such as *Trimastix pyriformis*.
PEPdb organizes the corresponding EST sequences and annotations, library information, redundancy and reading quality, and presents this information in a web-acessible form. Annotation is conducts automatically by the in-house developed tool AutoFact, which assigns information on gene and product name, molecular function, enzymatic pathway and gene ontology terms. Pathways can be queried, displayed and compared using the Pathway Tool software system (PD. Karp et al, BRG).
Newly generated data are initially stored in a proprietary database for curation and annotation, and subsequently made accessible to the wider research community (PEPdbPub at http://amoebidia.bcm.umontreal.ca/public/pepdb/agrm.php).
Future releases of the database will also include sequence data from other relevant eukaryotes and related information such as taxonomy.

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LITTLE WETLANDS IN THE BIG CITY: MICROBIAL MOLECULAR BIODIVERSITY AMONG AND WITHIN URBAN AND NON-URBAN GIS CHARACTERIZED VERNAL POOLS OF NORTHEASTERN OHIO.

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Due to human activities, snowmelt and runoff from urbanized areas likely contains dissimilar pollutants (i.e., deicing salts, heavy metal sediment, petroleum, lawn pesticides), as compared to better studied agricultural runoff (i.e. fertilizers, crop pesticides). Taxa rich vernal pools, largely recharged by undiluted runoff and common to both natural and urban landscapes, are thus attractive aquatic systems by which to examine the effects of on-going urbanization. Although highly impacted (i.e. the infamous “Burning River”), Ohio’s Cuyahoga River watershed still retains large and relatively pristine park areas (Cuyahoga National Park; Cleveland Metro-Parks), offering extreme comparatives of human activity. In this study, water quality of discrete vernal pools (n=30), influenced by differing degrees of urbanization, industrialization, and agriculture (as determined by GIS), was compared. Environmental genomic techniques, adapted to assess *in situ* biodiversity of algal, cyanobacterial, and fungal constituents within and among select vernal pools, will be presented so to illustrate on-going efforts to relate vernal pool biodiversity to urbanization.

PHYLOGENETIC RELATIONSHIPS OF THE CERAMIACEAE (CERAMIALES, RHODOPHYTA) BASED ON NUCLEAR SSU rDNA SEQUENCE DATA.

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The aim of the current investigation was to test the general convention that the Ceramiaceae is paraphyletic and the established hypotheses regarding subfamilial taxonomy. Phylogenetic relationships among 69 ceramialian (including 51 ceramiacean, six dasyacean, seven delesseriacean, five rhodomelacean algae) and 15 outgroup taxa (including eight related orders of the florideophycean algae) based on nuclear small subunit (SSU) rDNA sequence data were completed. Our results were consistent with the notion that the Ceramiaceae is paraphyletic leading to the Dasyaceae, Delesseriaceae and Rhodomelaceae. Although our analyses partially supported the subfamilies Ceramioideae (including tribes Aniththamnieae, Ceramieae, Dohrniellieae, Heterothamnieae and Pterothamnieae), Compsothamnioideae (consisting of the Compsothamnieae, Dasyphileae, Griffithsieae, Monosporeae, Ptiloteae, Spermothamnieae, Sphondylothamnieae, Spongoclonieae and Wrangelieae) and Crouanioideae (containing the Callithamnieae, Crouanieae and Rhodocallieae), they presented a far more complex scenario in comparison with the established proposals. These taxonomic issues and phylogenetic relationships among the Ceramiaceae based on female anatomical features will be introduced.

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AN OVERVIEW OF THE AQUANET INTEGRATED MULTI-TROPHIC (SALMON-KELP-MUSSEL) AQUACULTURE PROJECT IN THE BAY OF FUNDY, NEW BRUNSWICK, CANADA.

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Integrated multi-trophic aquaculture holds great potential for improving the sustainability of aquaculture. Based on an age-old, common sense, farming practice, the by-products (wastes) from one species become inputs for another: fed aquaculture (fish or shrimp) is combined with extractive inorganic aquaculture (seaweed) and extractive organic aquaculture (shellfish). With the support of AquaNet, the Network of Centres of Excellence for Aquaculture in Canada, we are developing such a system at an industrial pilot

scale by co-cultivating salmon (*Salmo salar*), kelp (*Laminaria saccharina*) and blue mussel (*Mytilus edulis*) at three aquaculture sites in the Bay of Fundy, Canada.
After three years of research, our findings support the establishment of integrated multi-trophic aquaculture systems for environmental sustainability (bioremediation), economic diversification (from fish filets to bioactive compounds) and social acceptability (better management practices). Innovative kelp culture techniques have been developed and improved both in the laboratory and at the aquaculture sites. Increased growth rates of kelps and mussels cultured in proximity to fish farms, compared to reference sites, reflect the increase in food availability and energy. Nutrient, biomass and oxygen levels are being monitored to model the bioremediation potential of an integrated multi-trophic aquaculture site. None of the therapeutants used in salmon aquaculture have been detected in kelps and mussels collected from the integrated sites over the three years of the study. A taste test at market size conducted on site grown versus reference mussels showed no discernable difference. A survey of aquaculture attitudes found that the general public is more negative towards current monoculture practices and, although relatively unfamiliar with the concept, feels positive that integration would be successful. We are now in the process of scaling-up experimental systems and working on an appropriate food safety regulatory and policy framework that will allow the development of commercial scale integrated operations.

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SYSTEMATICS OF THE RED ALGAL GENUS, *MEIODISCUS*.

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Meiodiscus is a genus of microscopic, marine red algae with an eventful taxonomic history. Based on morphological, biochemical and life history attributes it was moved from the Acrochaetiales to the Rhodophysemataceae, Palmariales. Molecular analysis of small subunit ribosomal DNA sequences confirmed the ordinal placement of *Meiodiscus*, however, the familial assignment is equivocal and requires further analysis. Here, we address the systematics of two morphologically similar *Meiodiscus* species, *M. concrescens* and *M. spetsbergensis*, and a species of *Rhodochorton* that has anatomical features consistent with inclusion in this genus. Sorting out the generic and familial relationships of these species, and the combined morphological and molecular rationale for their classification, are discussed. Features studied include cell dimensions, anatomy of tetrasporangia, presence or absence of cell fusions, and molecular sequencing of large subunit ribosomal DNA. Preliminary molecular results indicate considerable variation within a single 'species', uncovering previously undescribed diversity within this relatively obscure but intriguing group of red algae.

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GROWTH OF EPILITHIC ALGAE RELATED TO ZEBRA MUSSEL DENSITIES IN THE HUDSON RIVER.

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When zebra mussels (*Dreissena polymorpha*) invade lakes and rivers, phytoplankton communities are dramatically altered due to mussel feeding activity. This response is well documented. Less is known, however, about interactions between zebra mussels and benthic algae. We studied two sites in the Hudson River with significantly different densities of mussels, hypothesizing that this difference would be reflected in the community composition of epilithic algae. Quantitative epilithic algal samples were obtained on clay and quartz tiles at sites having either high (24,000 m^{-2}) or low (270 m^{-2}) *D. polymorpha*

densities. Tiles were placed in the river for one month during the summer of 2004. Tiles from the low-mussel-density-site (MC) contained roughly twice the amount of chlorophyll-*a* and particulate organic matter as those from the high-mussel-density-site (NP). Microscopic examination of algae colonizing the artificial substrates showed significantly higher densities of filamentous chlorophytes and cyanobacteria at MC, whereas NP tiles were dominated by both diatoms and a xanthophyte alga similar in appearance to members of the genus *Characiopsis*. In general, we observed a clear difference between the epilithic algal assemblages at the two sites. Only further study will permit us to determine whether this difference is the result of zebra mussel impact or, alternatively, whether the epilithic algae themselves somehow control mussel colonization.

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USING ALGAE TO EVALUATE THE CONDITION OF MAINE'S STREAMS & RIVERS.

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The Maine Department of Environmental Protection (MDEP) started sampling benthic algae from streams and rivers in 1999. To date, MDEP and its partners have performed 238 sample events from 176 sample locations across the state. MDEP targeted streams and rivers ranging from those with minimally disturbed watersheds to those with urban watersheds. The purpose of the sampling is to identify attributes of benthic algal communities that are reliable indicators of stream condition. MDEP intends to use the information to build a predictive model to help determine if streams and rivers attain their aquatic life criteria, which were assigned by the State Legislature. In addition, the U.S. Environmental Protection Agency is requiring states to adopt nutrient criteria for streams and rivers. MDEP hopes to use the algal data to help establish ecologically meaningful nutrient criteria. Over 840 taxa have been identified from the samples, including 560 Bacillariophyceae, 171 Chlorophyta, 89 Cyanophyta, and fewer taxa from other groups. Through preliminary analysis, MDEP has identified several algal community attributes that relate to human disturbance and nutrient enrichment. MDEP has identified some diatoms that may be useful as indicator taxa.

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DEVELOPMENT OF A RECIRCULATING INTEGRATED AQUACULTURE SYSTEM USING *PORPHYRA* FOR BIOREMEDIATION OF MARINE FINFISH EFFLUENT.

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The development of environmentally sound sustainable practices within the growing United States aquaculture industry is essential. The release of high levels of nutrients from aquaculture effluent into the marine environment can lead to toxic algal blooms and the growth of opportunistic seaweeds. The goal of integrated aquaculture is to use an economically valuable plant such as *Porphyra* to filter the effluent, effectively harnessing the nutrients to increase plant productivity. We have developed two modular integrated recirculating systems in which fish and nori are grown in separate 3600 L tanks within a greenhouse. Each system can operate on static, flow-through or recirculating mode. These systems have been used to measure the nitrogen uptake, growth and pigment production of several native *Porphyra*

species (*P. umbilicalis*, *P. amplissima* and *P. linearis*). Nitrogen uptake, growth and pigment production rates were comparable to previous studies. We have also measured the feed conversion rates, nitrogen production and growth of black sea bass (*Centropristis striata*) and Atlantic cod (*Gadus morhua*). From these measurements we generated a model to predict the nutrient levels that should be maintained for a range of seaweed:fish biomass ratios. The model was subsequently tested by adjusting the biomass ratio and monitoring nitrogen levels. Trial runs in recirculating mode successfully demonstrated that *Porphyra* was effective in maintaining low nitrogen levels in the system.

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FRESHWATER "GREEN TIDES": NUISANCE *CLADOPHORA* IN URBAN STREAMS.

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The filamentous green alga *Cladophora* has become a nuisance in local urban streams as well as in Lake Michigan over the past decade. In streams, it exhibits a seasonal change in morphology that appears correlated with water velocity and temperature. *Cladophora* over-winters as a basal crust, exhibits a highly branched morphology in spring and transitions into extremely long, sparsely branched filaments in early summer. Filaments may become encrusted with calcium carbonate under certain conditions. These concrete lined storm channels and restored streams lined with fresh limestone cobble provide an excellent substrate for *Cladophora* colonization. Such habitats, characterized by elevated nutrient supplies and extreme light intensity, become completely dominated by *Cladophora* in early summer, with nearly 100% spatial cover and biomass in excess of 160 mg per cm^2 dry weight (800 mg per cm^2 wet weigh). *Cladophora* biomass subsequently declines in mid-summer but there is a renewal of growth in early fall. *Cladophora* is an ecological generalist in these streams. It creates a food web bottleneck by producing a very large biomass which effectively captures all available resources (substrate, nutrients, light) but is largely inedible. The tremendous abundance of this alga reduces the aesthetic appeal of streams to urban residents. Photosynthesis and respiration by these substantial growths of *Cladophora* in the streams generates diurnal fluctuations in pH between 7.5-9.5 and dissolved oxygen fluctuations between 0-25mg/L (0-300% saturation) causing severe physiological stress for fish, invertebrates and other algae. Current research involves linking *Cladophora* morphology with photosynthetic characteristics, physiological condition, growth rates, natural reproduction and biomechanical studies of water velocities required to dislodge the filaments from the substrate.

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DEVELOPMENT OF AN ALGAL DIETARY SUPPLEMENT FOR DAIRY COWS ENRICHED IN THE OMEGA-3 FATTY ACIDS DHA & EPA.

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The objective of the research is to locate, acquire, culture and analyze algal species that have high levels of the omega-3 fatty acids DHA & EPA. Several culture trials with a wide range of algae are preformed under various environmental conditions (temperature, salinity, irradiance, light quality, photoperiod, culture phase, etc.) to maximize these important nutritional components. The omega-3 fatty acids, DHA & EPA, have been found to have an extremely beneficial effect in over 20 common human diseases. This research is an important component in a larger, five year research project in which we are developing an

enriched omega-3 fatty acid DHA & EPA dietary supplement, using algae, for dairy cows and tracking the incorporation of DHA & EPA into milk for human consumption.

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THE DEVELOPMENT OF ENVIRONMENTAL GENOMIC TECHNIQUES TO DETECT *IN SITU* MICROBIAL BROWN ALGAL LIFE-STAGES.

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Nearshore communities of polar and temperate zones are largely dominated by sporophytic life-stages of brown algae (Phaeophyceae). Likely due to their cryptic nature, brown algal microbial life-stage (meiospore, gametophyte, juvenile sporophyte) communities are poorly characterized, despite their probable importance to overall species distributions andpopulation persistence/recovery within variable environments. To overcome this deficiency, we are developing environmental genomic approaches to identify microbial brown algae in situ, as well as assess intertidal community composition and species distribution patterns. Successful application of molecular techniques largely depends on initial DNA extraction procedures that consistently yield high quality DNA from natural substrates. By comparing DNA yields, purity and capability of PCR amplification (eukaryotic 18S; algal ribosomal ITS1; phaeophytan mitochondrial CO1) four different DNA extraction procedures and sampling techniques were assessed. Tests were performed on naturally occurring substrate samples (granite-basalt composite) collected from the intertidal zones of western Vancouver Island, Canada. Preliminary analyses of *in situ* microbial brown algal life-stages detected on sampled intertidal substrates, which utilized our optimized DNA extraction procedure and DGGE (Denaturing Gradient Gel Electrophoresis), will also be presented.

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GLUTAMINE SYNTHETASE PHYLOGENY IN RHODOPHYTES.

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Glutamine synthetase (GS) is an essential enzyme catalyzing the ATP-dependent formation of glutamine from glutamate. In photosynthetic eukaryotes examined to date, a minimum of two nuclear encoded GS isoenzymes are present, compartmentalized in the cytosol and the chloroplast. Although GS has been broadly characterized from vascular plants, data from other photosynthetic eukaryotes are sparse. In rhodophytes little is known about GS isoenzyme expression or the regulation of nitrogen metabolism. In this study, we examined the phylogenetic relationships of GSII genes within the rhodophytes. GSII sequences were retrieved from EST databases and genome projects. The sequence from *Compsopogon coeruleus* was generated by PCR amplification of genomic DNA and sequenced. Maximum parsimony analysis consistently yielded trees in which rhodophytes were paraphyletic. Members of the Bangiales (*Porphyra*) and Compsopogonales (*Compsopogon*) formed one clade while the Gelidiales (*Gelidium*) and Cyanidiales (*Cyanidioschizon* and *Galdieria*) formed a separate clade. Both of the rhodophyte clades exhibited a sister association with the green plants. The topology of the tree drawn from the GSII phylogeny is not congruent with other phylogenetic analyses. One explanation for the incongruence is the presence of multiple GSII genes in rhodophytes. The analysis of the complete ORF's of GSII from *Gelidium*, *Galdieria* and *Cyanidioschizon* suggest that these are localized in cytoplasm. The partial GSII sequence obtained from *Porphyra* and *Compsopogon* do not include the N-terminal regions required for the determination of cellular compartmentalization. It is thus unknown whether the two clades represented here are derived from orthologous sequences. Our immediate goal is to increase taxon sampling within

the rhodophytes and to identify genes encoding different isoforms within those taxa. This will contribute to our understanding of the evolution of GSII and the regulation of nitrogen assimilating enzymes in rhodophytes.

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CALCIUM CHANNEL BLOCKERS INHIBIT THE ACETATE CHEMORESPONSE OF THE COLONIAL GREEN ALGA *ASTREPHOMENE GUBERNACULIFERA*.

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Cells communicate with each other and the environment through cell signaling. One common signaling pathway involves heterotrimeric G-proteins and a subsequent modification of calcium channels. The colonial green alga, *Astrephomene gubernaculifera,* shows a chemotactic response towards acetate, and it has been hypothesized that this is a result of specific receptor-mediated signaling events using this pathway. Here we used substances that inhibit the calcium channels and thus the flow of calcium ions into the cytosol. Colonies were grown for approximately 48 hours in HesA media. They were then washed three times in a Hes media containing no acetate, or nitrate, and containing 0.0225 mM free calcium. The colonies were then suspended in the wash media containing the calcium channel blockers diltaziem, ruthenium red, or verapamil. The effectiveness of the antagonists with respect to blocking the accumulation response was determined by observing a reduction in size and strength of the accumulation cloud upon addition of a 10 µL aliquot of 1mM acetate. Ruthenium Red inhibited the response at 10µM, completely blocked the response at 100µM, and reduced the duration of the response at 1µM. Verapamil inhibited the response at 250µM, completely blocked the response at 500µM, and significantly reduced the duration of the response at 25µM. Diltiazem inhibited the response at 400 µM, completely blocked the response at 600 µM, and significantly reduced the duration of the response at 200 µM. Thus, all three blockers inhibit the chemoresponse. Blocking the chemoresponse does not halt motility. We are presently analyzing the kinetics of swimming in the presence and absence of inhibitors to compare the swimming speeds and rates of turning. Ruthenium red and verapamil sensitivity is generally similar to that required to inhibit Ca^{2+} sensitivity in the unicellular alga *Chlamydomonas reinhardtii* (Ermilova, et al, 1998, Biologia Bratislava 53: 577).

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A COMPARATIVE GENETIC ANALYSIS OF THE GREEN DINOFLAGELLATES *GYMNODINIUM CHLOROPHORUM* AND *LEPIDODINIUM VIRIDE*.

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The dominant type of dinoflagellates has plastids containing chlorophyll c_2 and peridinin as main accessory photosynthetic pigments. These plastids were acquired from a red alga by secondary endosymbiosis. In a small number of dinoflagellate species, this original type of plastid has been substituted by other plastids, as indicated by their specific ultrastructure and photosynthetic pigmentation. Among these species, some of them have green, chlorophyll *b*-containing, plastids. We carried out a molecular genetics comparison on the two green dinoflagellate species cultured to date, *Lepidodinium*

viride and *Gymnodinium chlorophorum*, in order to determine their phylogenetic relationship and infer the green algal origin of their chloroplasts. The 18S rDNA sequences in the two species are identical, suggesting that they diverged very recently. Significant variations are observed, however, in the ribosomal intergenic spacers (ITS1 and ITS2) and the D1-D3 region of the 28S rDNA. The two species appear to contain a single 18S rDNA ribotype, suggesting that the organisms contain true plastids but not an endosymbiotic nucleomorph. This is in contrast to that suggested from ultrastructural analyses in the original descriptions of the two species. In *G. chlorophorum*, using pulsed-field gel electrophoresis and Southern blot analyses, we revealed a long plastid genome of ~100 kb in size. An ongoing phylogenetic analysis is being carried out to infer the green algal origin of plastids in the two species, by sequencing the plastid encoded genes *psbA*, *psbC*, *psaB* and *rbcL*. In *G. chlorophorum*, phylogenetic analyses of plastid *rbcL* and *psbA* genes indicate that the plastid was not derived from a prasinophyte. More likely, this plastid was obtained by secondary endosymbiosis of a modern chlorophyte, either from the Chlorophyceae or the Trebouxiophyceae. The comparison of the *psbA* and *psbC* genes sequences between the two species already revealed a huge genetic difference (> 10 %). We are investigating whether this indicates a different origin of chloroplasts or an extremely rapid rate of evolution happening in these organelle genomes.

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HISTONES AND HISTONE-LIKE PROTEINS IN DINOFLAGELLATES.

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Dinoflagellates exhibit a unique nuclear cell biology. Their permanent nuclear membrane surrounds immense genomes (often distributed among hundreds of chromosomes) that range in size from 1-71 times of that of humans. These massive genomes are comprised of DNA that appears to completely lack nucleosomes, the DNA packaging complexes comprised of histone proteins found in all other eukaryotes. Dinoflagellates are thought to have lost histones, instead their chromosomes are associated with histone-like proteins similar to homologs in bacteria. Analysis of an extensive EST data set from the dinoflagellate *Alexandrium tamarense* identified a set of the expected histone-like proteins but also resulted in the discovery of transcripts encoding chromalveolate-like histone H2A.X and H2A.Z. These histone proteins contain unique N-terminal extensions and tertiary structure predictions for the remainder of the sequence show high similarity to the crystal structure of histone H2A. We speculate these histones may have been retained for specialized roles, such as double-stranded DNA break repair.

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ISOLATION OF PCNA IN THE RED-TIDE DINOFLAGELLATE *AKASHIWO SANGUINEA* AND DEVELOPMENT OF A REAL-TIME PCR ASSAY TO QUANTIFY THIS SPECIES IN LONG ISLAND SOUND.

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Akashiwo sanguinea is a red-tide dinoflagellate species linked to fish kills. A molecular technique to allow identification and quantification of its abundance and estimation of its in situ growth rate would be very useful for monitoring the dynamics of this species and predicting formation of its blooms. Toward the goal to develop such a technique, a gene coding for proliferating cell nuclear antigen (PCNA) was cloned from *A. sanguinea* recently isolated from Long Island Sound. The high sequence variability of this gene shows high potential to allow design of species-specific primers. Furthermore, we found a high copy

number of this gene (about a hundred copies per cell), promising high sensitivity of a gene probe. A set of primers were designed and tested, yielding a pair of highly specific and sensitive ones. With this primer set, *A. sanguinea* abundance in Long Island Sound (LIS) was investigated. Seasonal samples were collected from seven stations throughout the sound and weekly samples taken from off the Avery Point campus of U Conn. The results and the potential utility of PCNA for monitoring *A. sanguinea* will be presented.

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PHYLOGENETIC RELATIONSHIPS AND IMPLICATIONS FOR THE CRYPTIC TAXA OF *PORPHYRA* (BANGIALES, RHODOPHYT) FROM KOREA BASED ON MORPHOLOGICAL AND MOLECULAR DATA.

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Nuclear SSU rDNA and plastid *rbc*L gene sequences as well as morphological comparisons were investigated for *Porphyra* species from Korea. 36 SSU rDNA and 65 *rbc*L are newly determined in this study. Molecular data from over 80 taxa of the Bangiales worldwide including previously published sequences, showed that monophyly for the genera *Bangia* and *Porphyra* is not supported, as in previous molecular studies. In addition, our data revealed the unexpected existence of seven cryptic taxa at least from East Sea of Korea, especially in the coasts of near Kangnung. Most of them have been misidentified as *P. yezoensis* or *P. pseudolinearis*, which shows a great variation in morphology. Two of them are similar to *P. yezoensis* in sharing conspicuous trichogynes, and other three of them are dioecious such as *P. pseudolinearis*. Another is similar to *P. katadae* with half-parted monoecious thallus, and the seventh taxon has a funnel-shape thallus such as *P. umbilicalis*. Our molecular data shows that Korean components of *Porphyra* are highly diverse in genetic by existence of five scattering ones among eight lineages of *Bangia* and *Porphyra*. The taxonomic issues and phylogenetic relationships of Korean *Porphyra* species among the Bangiales will be discussed.

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CHRYSOPHYTE RESTING STAGES: A POWERFUL TOOL FOR RECONSTRUCTING CLIMATE FROM ALPINE LAKE SEDIMENTS.

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Chrysophyte algae (classes Chrysophyceae and Synurophyceae) produce resistant siliceous resting stages (stomatocysts) that are known to be powerful indicators of past environmental conditions. The objective of this study was to assess their strength for climate reconstruction. Stomatocysts were collected using sediment traps exposed in 45 mountain lakes (1502 – 2309 m a.s.l., Austrian Alps). Bi-hourly water-temperature measurements were used for determination of ice-on, ice-off, spring and autumn mixing. Canonical correspondence analyses revealed that the stomatocyst assemblages were related to the date of spring mixing. Date of spring mixing is controlled by winter/spring air temperature. We developed a weighted averaging – partial least squares (WA-PLS) stomatocyst/date-of-spring-mixing regression and calibration model (R2boot = 0.85), and reconstructed dates of spring mixing for Lake Jezero v Ledvici

(1824 m a.s.l., Slovenian Alps) from 1842 to 1996 AD. The sample-specific standard error of prediction corresponded to 0.6°C – 1.0°C. Despite very poor fits with the training set, reconstructed dates of spring mixing were significantly correlated with the mean March – April air temperature (R = -0.35), which is known to drive ice-out dates. They were in excellent agreement with the last glacial advances during the Little Ice Age.

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SEXUAL REPRODUCTION IN *DITYLUM BRIGHTWELLII*.

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Diatoms are obligately sexual organisms, but sexual reproduction is rarely seen in nature. It is important to the understanding of diatom ecology, population dynamics, and evolution to know the mating systems of diatoms and the frequency of sexual reproduction in nature. This study combines laboratory experiments and field studies to determine the mating system and timing of sexual reproduction in *Ditylum brightwellii*, a marine centric diatom. Field samples, and single cell isolates were collected from Wadsworth Cove, Castine, Maine. Gametogenesis was correlated with cell size. The smallest clones tended to produce spermatagonia; the intermediate-sized clones produced oogonia; the largest clones did not undergo gametogenesis. However, *D. brightwellii* is homothallic, and capable of self-fertilization. The timing of gametogenesis within and between clones determined whether out-crossing or self-fertilization predominated. Temporal variation in gametogenesis may provide a mechanism for *D. brightwellii* to avoid selfing. Samples from natural populations of *D. brightwellii* indicated that sexual reproduction occurred during the autumn of 2004. Spermatagonia were observed at low frequencies (0.4 – 1.0%) from late August to mid-October, and we observed a general trend of increasing cell size during this period. The sizes of the largest cells and spermatagonia from the field samples were within the same size range as the initial cells and spermatagonia produced in laboratory experiments. (Supported by NSF OCE-99043 to S.H. Brawley. Special thanks to Prof. David Mann for advice and discussion.)

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AN EXAMINATION OF THE EVOLUTION AND DIVERSIFICATION OF LIGHT-HARVESTING COMPLEXES IN *EUGLENA GRACILIS*.

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Light harvesting complexes (LHCs) are found in all photosynthetic organisms, and are responsible for the capture of light energy, and its transfer to the photosynthetic reaction centres. The LHCs are encoded by a nuclear, multi-gene family, but are located within the thylakoid membrane of chloroplasts where they bind carotenoids and chlorophylls. Like other protists, *Euglena* acquired a plastid secondarily through an endosymbiotic event with a photosynthetic eukaryote. These organisms are particularly interesting to study because during the evolution of the secondary plastid, LHCs and other photosynthetic genes and regulatory components must have been transferred to the host's nuclear genome. They therefore provide a unique opportunity to study the diversity and evolution of the LHC multi-gene family during a plastid acquisition event.
There are two main objectives to this project: 1) to examine the diversification and evolution of LHC proteins in the protist *Euglena gracilis*, and 2) Examine the fine structure of the unsually large *E. gracilis* mRNA molecules that are translated into polyproteins, which are then post-translationally cleaved into

multiple, distinct LHC proteins. As a part of the Protist EST Program (PEP), over 10 000 ESTs have been generated and analysed in an effort understand the process of secondary plastid evolution, and in particular, evolution of the Lhc gene family.
Phylogenetic analyses have been performed on the LHC homologues in order to study the diversity of the LHC gene family. Preliminary data suggests that though Euglena LHC proteins can be divided in to LHCI and LHCII, as in plants and algae, they lack the orthologs of most of the green algal/plant antennae complexes, with the exception of CP29. LHCI-related genes, in particular, seem to have undergone extensive duplication and divergence. This would imply that there are significant differences in the function and regulation of light harvesting in these chlorophyll a/b-containing protists compared to green algae with similar pigmentation. ..
Most of the LHCs were clustered as polyprotein-encoding mRNAs of up to 3500 nt, with the exception of CP29, which was found as a monocistronic mRNA. Within the polyproteins, the individual Lhc subunits are separated to each other by conserved decapeptide linkers. The complexity of the *Euglena* Lhc family and its functional implications will be discussed.

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RESOLVING SPECIES LIMITS FOR THE BROWN ALGAL GENUS *FUCUS* IN THE NORTHEAST PACIFIC.

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In the Northeast Pacific there are two morphologically distinct species of the genus *Fucus*: *F. spiralis* and *F. gardneri*. These species grow in a continuous band over the mid to high intertidal zone of rocky shorelines. *Fucus spiralis* is found in the high intertidal whereas *F. gardneri* inhabits the mid intertidal. Between unequivocal *F. spiralis* and *F. gardneri* is a zone inhabited by individuals that variously display morphological characteristics of both species. This intermediate zone may be the result of phenotypic plasticity of one or both species, hybridization between the two species, or some combination of these events. Alternatively, the individuals may simply be morphological expressions of a single species at varying heights in the intertidal.
We will test these hypotheses with molecular tools. First, we will use genetic barcoding (ca.600bp region of the cytochrome oxidase I gene) to establish genetic limits of all of the 'species' in this genus. We will then construct phylogenies of the entire genus based on nuclear (internal transcribed spacer; ITS) sequences and compare these to the mitochondrial results. If the results disagree, hybridization may be occurring between some species. We will then use these same marker systems to evaluate *Fucus* species in the Northeast Pacific on a finer scale, applying them to multiple individuals across vertical gradients of the intertidal from four geographically diverse regions. In addition, PCR primers specific to *F. spiralis* and *F. gardneri* will be designed for the nuclear markers to enable hybrids to be unequivocally identified. The molecular work will be combined with morphological observations to resolve species limits for *Fucus* in the Northeast Pacific.

GENOMIC DIVERSITY AND CHROMOSOME STRUCTURE OF CRYPTOMONAD NUCLEOMORPHS.

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The cryptomonads are a highly unusual group of eukaryotic microbes that have acquired photosynthesis through a process called secondary endosymbiosis. This occurs when a phagotrophic eukaryote engulfs a photosynthetic eukaryote and retains its plastid (chloroplast). Cryptomonads are unusual in that they retain the nucleus of their eukaryotic endosymbiont in a highly reduced form, called a nucleomorph. In all cryptomonads investigated thus far, the nucleomorph genome is spread across three chromosomes of varying size. Our objective is to use a comparative genomics approach to study the evolution of nucleomorph genomes in a wide range of cryptomonad species. Here we present preliminary data, showing the diversity of cryptomonad nucleomorph chromosome and genome sizes, and examining in detail the sub-terminal region of nucleomorph chromosomes in two closely related species. Our results indicate that (1) nucleomorph genomes can range from ~450 Kilobases (Kb) to ~750 Kb in cryptomonads, (2) Inter-species nucleomorph chromosome size is highly variable, (3) the presence of ribosomal DNA (rDNA) operons adjacent to the telomere is a common feature of cryptomonad nucleomorph chromosomes, (4) the rDNA operon is flanked by a lengthy region of non-coding DNA, and (5) this non-coding region is highly conserved amongst different chromosomes within the same organism, but highly variable between different species.

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A NEW KELP (LAMINARIALES, PHAEOPHYCEAE) PHYLOGENY ELUCIDATED WITH MOLECULAR DATA.

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Members of the order Laminariales, commonly called kelp, are some of the most extensively studied and widely recognized macroalgae in the world. However, despite their conspicuous nature and the focused attention of the scientific community, taxonomic relationships among genera of the Laminariales remain unresolved. Recent publications have used sequence data to change significantly the taxonomic constitution of the "primitive" families traditionally recognized in the Laminariales. The three "advanced" families, conversely, have remained virtually unchanged since 1925 despite sequence data over a decade ago indicating that the advanced families are polyphyletic. Two phylogenetic hypotheses have emerged through the literature, differing mainly in the number of clades (families) recognized and the relationships among them, but both lacked sufficient support to justify sweeping taxonomic modifications. In order to reconcile these hypotheses, we have sequenced genes from the nuclear, chloroplast and mitochondrial genomes and used distance, likelihood and Bayesian analyses to create a robust phylogeny for the advanced families of the Laminariales. Our results indicate that sequence analyses artifacts have compromised phylogenetic resolution in previous studies. A contemporary classification for the "advanced" kelp will be proposed based on our findings.

HOW MANY GENES ARE REQUIRED TO INFER DEEP PHYLOGENIES – THE CALL FOR PHYLOGENOMICS.

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Key events in eukaryotic evolution such as the introduction of photosynthesis *via* endosymbiosis with a Cyanobacterium, or the divergence of major lineages such as the Opisthokonts, Stramenopiles or Ciliates, occurred more than a billion years ago. Despite the plethora of published molecular phylogenies, solid phylogenetic evidence is usually lacking because most analyses are based on only a single, or few genes. To address this issue, we will discuss the predictive power of phylogenomic analyses with large sets of mitochondrial protein-coding genes, and nuclear EST data. We find that statistically significant support for deep divergences usually requires (i) many more genes than are available from mitochondrial genomes; (ii) broad taxon sampling from species that evolve at moderate rates; (iii) the use of sophisticated phylogenetic inference methods such as separate maximum likelihood or Bayesian analysis; (iv) careful elimination of oversaturated sites in the sequence alignments, and (v) occasionally, the elimination of species that evolve at highly accelerated rate and that retain insufficient phylogenetic signal to be detected with currently available methodology.

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A QUANTITATIVE COMPARISON OF CYTOPLASMIC ORGANIZATION FROM THE APICAL TIP AND MATURE STIPE REGION OF THE COENOCYTE, *CAULERPA MEXICANA*

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Organelle and cell wall trabeculae volume percents (Vv) within the giant coenocytic green alga, *Caulerpa mexicana* (Sander) J. Agardh, were quantified using stereology methods. Samples were taken from six regions of the stoloniferous stipe; six within the apical tip from 0-10 mm and one region approximately 70-150 mm from the tip (mature region) from 11 separate plants. Light microscopy was used to make Vv measurements of cytoplasmic, vacuolar, trabecular, and plastid volume. Using Tukey-Kramer Multiple Comparison tests for Vv, we found a significant decrease of cytoplasmic Vv from the tip through 10 mm, an increase in trabeculae Vv from tip to 5 mm (followed by a decrease in 6-10 and mature regions), and an increase in vacuole Vv from the tip to 10 mm. In addition, the tip vacuole Vv was significantly lower than the samples of the mature regions. Based on significant differences of the parameters measured we can recognize three separate regions of cytoplasmic differentiation of the stipe in *C. mexicana.* The three regions we have identified are: a) the tip to approximately 3 mm; b) from 4-10 mm; and c) the mature region. Further studies with the transmission electron microscope will address more details of cytological structure in each of the zones described in this study.

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MOLECULAR SYSTEMATICS OF THE FLORIDEOPHYCEAE (RHODOPHYTA) USING NUCLEAR LARGE AND SMALL SUBUNIT rDNA REVISITED WITH BAYES

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Ribosomal DNA data have been used extensively to infer red algal phylogenies for more than a decade. Thus far, the most comprehensive study to infer ordinal relationships among Florideophyceae was published by Harper and Saunders in 2001. In that study, the data, which includes LSU and SSU sequences for 45 taxa representing most of the currently recognized florideophyte orders, had been analyzed with maximum likelihood using the Kimura 2 parameter model of DNA evolution.
We reanalyzed their SSU-LSU combined data set exploring the effects of several models of DNA evolution, as well as partitioning on tree likelihoods and posterior probabilities within a Bayesian framework. Over the conditions tested, the use of more complex models of DNA evolution within the Bayesian framework improved tree scores and corrected previous anomalies. Our results suggest that the use of complex models of evolution within the Bayesian framework perform better for analyses of ribosomal DNA. To test further interordinal relationships within the Florideophyceae, we extended the SSU-LSU alignment of Harper and Saunders to 75 taxa, and additionally developed the nuclear protein-coding gene EF2 for these same taxa. These three regions were analyzed separately and in combination using distance, parsimony, and Bayesian approaches. New insights on florideophycean phylogeny will be discussed.

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ASSESSING THE PATTERN OF GEOGRAPHIC VARIATION IN THE GREEN SYMBIONT OF THE TEMPERATE SEA ANEMONE *ANTHOPLEURA XANTHOGRAMMICA*.

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In the North Pacific intertidal the sea anemones *Anthopleura xanthogrammica* and *A. elegantissima* can each form symbioses with two distinct photosynthetic protists, a green alga (Chlorophyta), and a better-known dinophyte belonging to the genus *Symbiodinium*. Previous studies of this dual symbiont system have focused on ecological questions dealing with the influence of light and temperature on carbon allocation and symbiont distribution. Populations of *Anthopleura* usually harbor dinophytes in southern latitudes and green symbionts at more northern latitudes (>43^{o}N). Previous genetic studies on the dinophyte symbiont of *A. elegantissima* found that southern-most populations can have a mixture of *Symbiodinium* species, whereas a single species of *Symbiodinium* is found in the northern-most range of the dinophyte symbiont form (LaJeunesse and Trench 2000). Recently, the phylogenetic position of the marine green alga inhabiting *A. elegantissima* was determined by comparing sequence data from the nuclear 18S rDNA and the plastid encoded rbcL genes. Though called "zoochlorellae" this symbiont is not a true *Chlorella* (*sensu* Huss et al. 1999) but is more closely related to *Trebouxia* and *Coccomyxa* (Lewis and Muller-Parker 2004). In order to compare the green symbionts in the two related anemone species, and assess the geographic pattern of genetic variation in the green symbionts of *A. xanthogrammica*, we have analyzed samples of anemones containing green symbionts from their known range along the North Pacific coast, including Alaska, Oregon and Washington. To avoid complications of amplifying anemone DNA, we initially targeted the plastid-encoded rbcL gene. While rbcL is a relatively slowly evolving gene, it contains variation among closely related species within *Bracteacoccus* (LL, unpub) and *Pediastrum* (McManus, unpub). RbcL gene sequences from the green symbionts in *A. xanthogrammica* are identical in sequence to those of the related anemone *A. elegantissima*. In addition, they show no variation across the geographic locations analyzed. Since the *Symbiodinium* geographic variants were identified using 18S and ITS2 rDNA data, we plan to collect data from more variable genes to further compare the pattern of geographic variation of zoochlorellae to that seen in *Symbiodinium* of *A. elegantissima*.

PORPHYRA FROM SEA TO FRESHWATER; OSMOREGULATION IN MARINE ALGAE.

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The development of a practical integrated seaweed culture system could bioremediate finfish hatchery effluents while producing a second commercial crop. Modifying marine algae, e.g. *Porphyra* spp. to bioremediate effluents from a freshwater fish hatchery may seem anomalous; however a commercial, cold freshwater alternative does not exist. The development of a zero salinity tolerant cultivar is the focus of both cultural and molecular investigations.

The development of a freshwater *Porphyra* cultivar, the singular goal of year one of a two-year USDA sponsored research effort, has been achieved. Thalli are presently growing at a greater rate, although not significantly, in ion-supplemented freshwater (0.06 ppt) as compared to the control of full strength seawater (32 ppt).

Calcium ions have been demonstrated to exert key regulatory effects on algae when exposed to low salinities. Previous reports suggest that: 1) calcium is a key element in determining algal survival at low salinities and 2) that genetic isolates of various seaweeds may possess alterations in distinct genes including Calcium Receptors (CaR) that provide them with the ability to adapt to the shifting salinities. CaRs have been shown to be the "master controller" of divalent calcium homeostasis in humans, flowering plants and green algae. Preliminary efforts have suggested similar molecules in *Porphyra*.

As a result of the progress made during the initial twelve months of the USDA grant, we increased the scope of the research plan to include both the gametophytic (as originally described in the grant proposal) and the sporophytic or conchocelis form of *Porphyra's* life history in the low salinity experimentation. Anticipated year two benefits include: expanding the cultivation scale, developing the ability to culture algae in divalent cation supplemented freshwater with finfish effluents, and investigate the molecular basis for algal osmoregulation.

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AN INVASIVE *PADINA* ON A BLEACHED CORAL SITE IN THE ANDAMAN SEA

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Phongsuwan documented a massive overgrowth of *Padina* on a bleached coral site in the Surin Islands in the Andaman Sea in 1997. The bleaching at this site was observed as early as 1991. This *Padina* population has maintained itself in varying but profuse amounts until the present time. Therefore, although it is a native genus, *Padina* has behaved invasively at this site. In an attempt to understand the invasive success of *Padina* we have compared the reproductive patterns of the Surin population with those of non-reef-associated populations from around Phuket Island, also in the Andaman Sea. There are no other reports in the literature of *Padina* as an invasive alga. Patterns of sexual and asexual stages from the Surin bleached coral site differ from those of populations from Phuket Island. Also, besides the fan shaped foliose morphology, *Padina* maintains a basal phase, more creeping in habit, from which numerous growing points emerge acting as a vegetative propagule. The invasive success of *Padina* populations can in part be attributed to this vegetative reproduction. This phase is less subject to wave action and, being in the interstices of the coral rock, is also less available to grazers. Any removal experiments designed to assess the impact of *Padina* on bleached coral recuperation have to take this phase into consideration because, as a remnant of the massive population it can regrow readily without sexual reproduction or asexual spore germination.

UNEXPECTED STRUCTURE AND EXPRESSION PATTERN OF PCNA IN THE DINOFLAGELLATE *PFIESTERIA PISCICIDA.*

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Proliferating cell nuclear antigen (PCNA) and its encoding gene have been identified in a wide range of organisms including several groups of microalgae. Although dinoflagellates are ecologically and economically important, their PCNA genes have not been studied. In this study, we analyzed *pcna* and its encoded protein from the putatively toxic dinoflagellate *Pfiesteria piscicida* (*Ppi_pcna*). Using RT-PCR and RLM-RACE methods, the coding region (777 bp) and the 5' and 3'-non-translated ends of *Ppi_pcna* cDNAs were isolated, with their 5'-end and coding regions essentially identical but 3'-endregions drastically different. The coding region was composed of 258 amino acid residues (aa), similar to that of vertebrate PCNA (260-262 aa) and typical plant PCNA (264 aa), and shorter than that of its alveolate relatives *Plasmodium* (274 aa, X68739 and *Toxoplasma* (316 aa, AF242301). PCR using genomic DNA as the template yielded multiple products, whose sequences revealed tandem repeat copies of *pcna* separated by unknown sequences. Using Real-Time PCR, we detected 41±7 copies of this gene in each *P. piscicida* cell. Reverse transcription Real-Time PCR indicated a fairly constant *pcna* mRNA level between exponential and stationary growth phases. Western blot analysis using a polyclonal antiserum raised against the *P. piscicida* PCNA revealed a slightly more PCNA per cell (about 1.5 times) in the exponential phase than the stationary growth phase. The results suggest that 1) *P. piscicida* and likely other dinoflagellates possess a typical *pcna*; 2) unlike in other eukaryotes examined so far, *pcna* occurs in multiple copies in *P. piscicida*, most likely as a result of rampant duplication; 3) regulation of the function of *P. piscicida* PCNA may reside at differential expression of different copies and posttranslational modification or intracellular localization; 4) utility of PCNA for *P. piscicida* growth rate studies needs to be assessed further.

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ELECTROPORATION OF *PORPHYRA YEZOENSIS* MONOSPORES.

Yen-Chun **Liu**, Andrew J. Cary and Donald P. Cheney. Biology Department, Northeastern University, Boston MA 02215

The red alga *Porphyra yezoensis* is widely cultivated and is one of the most extensively studied of the macroalgae. Our lab is interested in understanding the biology of Porphyra, as well as developing it as a potential resource for biotechnological applications. In order to use many of the current molecular genetic and genomic approaches it is important to have a high efficiency genetic transformation system for *Porphyra*. Our lab has had previous success using the soil pathogen *Agrobacterium tumefaciens* to introduce new genes into *Porphyra* but have found that transformants are produced at a low frequency. Recently a method was developed that allows the mass production of monospores in culture (Hiroyuki M. et al., Journal of Applied Phycology 15:345, 2003). We are working to develop a high efficiency transformation system though the electroporation of induced *Porphyra* monospores. Since it is unclear how robust reporter genes from green plant or animal constructs are expressed in *Porphyra* we evaluate monospore electroporation using fluorescent dye accumulation as an assay. We have achieved a high frequency of dye loading by optimization of the electroporation and growth conditions. We have also grown the electroporated monospores back to full blades. In parallel to the electroporation experiments we are developing new gene expression cassettes based on *Porphyra* sequence information in order to introduce new genes into the monospores.

INVESTIGATIONS OF THE TOLERANCE ABILITY OF *PORPHYRA* TO SURVIVE EXTREME ENVIRONMENTAL STRESSES.

Yen-Chun **Liu**, Tim Hogan, Andy Cary, Angela Silvestro Leslie Graham, and Donald Cheney. Biology Department, Northeastern University, Boston, MA 02115.

The intertidal red macroalga *Porphyra* lives in an environment in which it is exposed to extremes in light and temperature on a tidal, daily, and seasonal basis. During the winter, for example, gametophyte blades experience extreme desiccation (with over 90% water loss) and freezing, while during the summer, blades experience extreme desiccation and temperatures over 25 ^{o}C. Because it has cogeners that live at different intertidal heights, as well as a sporophyte (Conchocelis) phase that is believed to live only subtidally, we think *Porphyra* presents a good model system to investigate how such seaweeds have adapted to extreme environmental stresses at the biochemical and molecular levels. This is an area that not much is known about in seaweeds (Davidson and Pearson, 1996, J. Phycology 32:197) but a great deal is understood in land plants. This poster will describe some of our preliminary efforts in this area. For example, we have some data comparing the polyunsaturated fatty acid (PUFA) composition of blades and Conchocelis with their relative ability to withstand freezing. Interestingly, we have found a significant difference in the PUFA composition in blades vs. Conchocelis, as well as an overall trend toward an increase in unsaturated fatty acids at low temperature. We are also looking at whether sugars and antioxidants (electron scrubbers) might be playing key roles in desiccation tolerance like they do in land plants. And finally, we have some information on the lower salinity limits for *Porphyra*, in both blades and Conchocelis. It is our hope that this poster may stimulate collaborative research interest in this area.

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AN INVENTORY OF SCALED CHRYSOPHYTES ALONG COASTAL MAINE, USA, AND THEIR RELATIONSHIPS TO ENVIRONMENTAL VARIABLES.

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The distributions of silica-scaled Chrysophyceae and Synurophyceae in 31 freshwater waterbodies from three regions located along the coast of Maine, USA, are described and related to chemical variables. Phytoplankton, periphyton, and surface sediments from each of the 31 sites were collected in June of 2002 and later analyzed extensively with both scanning electron microscopy (SEM) and light microscopy (LM) for scaled chrysophytes. In addition, water samples were used to measure a variety of chemical characteristics, including specific conductivity, pH, alkalinity, total phosphorus, total nitrogen, chloride, sulfate and base cation concentrations. The three studied areas were the China Lakes Region along the southern coast, Acadia National Park of the middle coast, and the Moosehorn National Wildlife Refuge along the northern coast. The waterbodies are of glacial origin and are characterized as dilute, oligotrophic, and low in alkalinity. Waterbodies in all three localities were found to be rich and abundant in scaled chrysophytes. To date, we have identified over 60 taxa of silica-scaled Chrysophyceae and Synurophyceae, representing six genera. The number of taxa found per lake ranged from 10 to 33 and observations include new records of many rarely reported species such as *Mallomonas guttata* f. *simplex* and *M. perfossa*. Dominating the flora were *Synura echinulata*, *S. petersenii*, *Chrysosphaerella longispina*, *Mallomonas caudata* and *Synura sphagnicola*. In addition, nine species of the genus *Spiniferomonas* were recorded. Waterbodies with the highest diversity and abundances of scaled chrysophytes can be characterized as moderately acidic, dilute and slightly humic, supporting findings from previous studies.

TOWARD RESOLUTION OF GREEN PLANT PHYLOGENY.

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The tree of life is inherently fractal. Look closely at one lineage of a phylogeny and it dissolves into many separate lineages, and so on. A great body of phylogenetic research has provided numerous tools applicable at particular, usually fairly constrained, scales. These tools have left many phylogenetic questions unanswered. We think these questions will remain unanswered until it is possible to do analyses across multiple scales. Our objective in this research is to resolve the primary pattern of evolutionary diversification among green plants. We believe that a solid backbone based on multiple classes of data from genomics to morphology will enable the integration of previous and ongoing studies of many more taxa into a comprehensive picture of green plant phylogeny. We have selected 50 taxa for analysis, with special reference to "difficult" problems in green plant phylogeny such as the origin and early diversification of bryophytes, the monophyly of the algal class Ulvophyceae, and the relationships among prasinophytes. For these taxa we are sequencing mitochondrial and chloroplast genomes and are constructing Bacterial Artificial Chromosome libraries for their nuclear genomes. We are having success with flow-cytometry mediated organelle sorting and rolling-circle DNA amplification to obtain organelle genomes relatively quickly and a considerable reduction in the amount of plant material required. Also, for these same taxa, we are assembling a comprehensive set of morphological and ultrastructural data. We intend to use these data to infer a "core" phylogeny, and develop computational tools and strategies that permit scaling across phylogenetic studies. Supported by five grants from the NSF Assembling the Tree of Life program. Project website: http://ucjeps.berkeley.edu/TreeofLife/.

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THE LARGEST GROUP OF ALGAE BEGINS TO MAKE SENSE: THE CONTRIBUTION OF GENOME STUDIES TO UNDERSTANDING DIATOM BIODIVERSITY.

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Even diatomists have usually underestimated the scale of the contribution made globally by diatoms to atmosphere–ocean fluxes and nutrient cycling, the number of diatom species, and the possibilities diatoms offer for monitoring present and past environments. This is a truly significant group of organisms. The recent sequencing and annotation of the *Thalassiosira pseudonana* genome by Armbrust et al. has provided new insights into diatom metabolism, e.g. the unexpected demonstration of a urea cycle, besides features likely to be characteristic of only a subset of diatoms, e.g. multiple chitinases and aspects of wall biochemistry. Another whole genome sequence, for *Phaeodactylum*, is under way, in the context of previous work demonstrating transformation of this organism. Diatom shells are intricate and can be used to derive many characters for taxonomy and phylogenetic reconstruction, leading by 1990 to a more developed (though not necessarily more accurate) classification than for possibly any other group of microbial eukaryotes. Since c. 1990, the classifications derived from morphological data have been tested using molecular data. Results indicate that at the species level, diatoms are grossly underclassified, supporting earlier conclusions from mating data and morphometrics. Cryptic species are likely abundant.

At higher levels, molecular data have provided good insights into family relationships, where morphology is equivocal because of extensive homoplasy. However, at ordinal/class levels, although some aspects of phylogeny are well established (e.g. monophyly of raphid diatoms, monophyly of pennates) and correlate well with other sources of information (cytological, reproductive, morphogenetic, morphological), many nodes have tantalizingly low support in each data-set. Multiple gene approaches may help (but what if diatom evolution has been dominated by a few ancient explosive radiations?). One extra caveat: lack of basic information about many taxa means that, even if the phylogeny is established, we won't be able to make much sense of it.

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USING MOLECULAR TOOLS TO RESOLVE DIVERSITY AND PHYLOGENETIC AFFINITIES OF SPECIES INCLUDED IN THE RED ALGAL GENUS *RHODYMENIA* FROM AUSTRALIA.

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The red algal family Rhodymeniaceae has had an inconsistent taxonomic history because of fluctuating emphases on varying anatomical characters. Traditionally based on vegetative characteristics including algae with either solid thalli or hollow thalli lacking periclinal filaments, the modern perspective focuses on reproductive anatomy and contains rhodymenialean algae with four-celled carpogonial branches, cruciate and intercalary tetrasporangia, and an absence of a *tela arachnoidea*. In this regard, most species of the type genus, *Rhodymenia*, have features consistent with the familial description. However, a few Australian species exhibit reproductive structures in conflict with this construct. The objective of this research is to resolve the phylogenetic affinities of all of the presently described species of *Rhodymenia* in Australia through molecular phylogenetic analyses of the large subunit ribosomal DNA. Our molecular data indicate that the species of *Rhodymenia* with anomalous reproductive characteristics belong to segregate genera. Specifically, *Rhodymenia stenoglossa*, *R. obtusa*, and some collections of *R. sonderi* group with other species of *Rhodymenia*, whereas *R. verucossa*, *R. cunneata*, and some collections of *R. sonderi* group more closely with the genus '*Halichrysis'*. A third distinct grouping from the *R. sonderi* collections groups with *Fryeella*. The taxonomic and phylogenetic consequences of this research will be discussed.

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FUZZY SPECIES BOUNDARIES IN HYDRODICTYACEAE (CHLOROPHYCEAE): CAN MOLECULES AND GROWTH STUDIES GIVE US 20/20 VISION?

Hilary A. **McManus** and Louise A. Lewis. Department of Ecology and Evolutionary Biology, University of Connecticut, 75 N. Eagleville Rd. Box U-43, Storrs, CT 06269, USA.

Robust phylogenetic trees offer insight to morphological variation within species, the validity of taxonomic designations, and evolution of morphological traits such as body forms. In addition, a phylogenetic tree provides a framework for the interpretation of morphological variation seen in response to differing environmental cues (phenotypic plasticity). Constructing a robust phylogenetic tree is dependent on several important factors, including adequate taxon sampling, obtaining data of sufficient variation, and including data from independent sources such as multiple genes and morphology. Our work on the systematics of the green algal family Hydrodictyaceae (Sphaeropleales, Chlorophyceae), and questions regarding species boundaries and morphological evolution, has involved a search for gene sequence data from multiple cellular compartments that could provide well resolved and well supported

nodes. This study has also demonstrated that increasing taxon sampling continues to impact the tree topology and our interpretations of species relationships. Our results thus far reveal a pattern of evolution from two-dimensional *Pediastrum* colony forms to three-dimensional *Hydrodictyon* and *Sorastrum* colony forms, and numerous difficulties in interpreting species boundaries within the genus *Pediastrum*. In conjunction with constructing a molecular phylogenetic tree, preliminary growth experiments on strains of *Pediastrum* have revealed morphological differences, in at least one strain, when colonies are grown in varying concentrations of growth medium. These changes in morphology may result in differing taxonomic designations, raising questions as to what morphological characteristics are taxonomically informative. Discussed are a comparison of the phylogenetic signal and resolution found in sequence data collected from three cellular compartments, the nucleus (26rRNA), chloroplast (*rbc*L) and mitochondrion (coxI), as well as what recent phylogenetic analyses and plasticity studies reveal about species boundaries and morphological evolution in the genus *Pediastrum*.

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AUTOGAMY IN *THALASSIOSIRA NORDENSKIOELDII* AND SELECTED OTHER MEMBERS OF THIS GENUS.

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The life history of diatoms is diplontic with propagation being primarily asexual where in many species population mean cell size declines until individual cells become sexually inducible. During sexual reproduction and resulting auxosporulation, expansion of the diploid zygote restores large cell size, whereby ensuring the survival of the population and species. The understanding of sexual reproduction in centric diatoms lags behind the pennates. This project has two goals: to determine sexual behaviour and gametogenesis in selected centric diatoms (via Epifluorescent Microscopy (EM), and to determine their reproductive cell fine structures using Scanning Electron Microscopy (SEM). Three *Thalassiosira* species (*T. nordenskioeldii*, *T. punctigera*, and *T. pseudonana*) were successfully induced. Early stages of auxosporulation were observed in all three species, in three clones *of T. nordenskioeldii*, triplicates of the same clone in *T. pseudonana* and in one clone of *T. punctigera*. In *T. pseudonana* and *T. punctigera* auxosporulation did not run its full course and initial cells were not observed in these clones. Spermatogenesis was absent in all our clones as neither spermatogonangia nor sperm nuclei were observed during the induction. Induction success varied between 16.75% and 40.62% in *T. nordenskioeldii*, was 12.33% in *T. punctigera* and was 21.53% on average in *T. pseudonana*.
EM observation of the nuclear behaviour during auxosporulation of *T. nordenskioeldii* confirmed that it reproduced autogamously. Autogamy in this species began with an unequal mitotic division and subsequent pervalvar expansion of the auxospore mother cell. The smaller cell often continued normal vegetative growth. In the auxospore mother cell, Meiosis I was acytokinetic and resulted in one viable haploid, and one pycnotic nucleus. Meiosis II resulted in the production of two viable haploid nuclei which fused to produce the diploid zygotic nucleus. Auxospore expansion, accompanied by the production of scales, was observed in cells both prior to nuclear fusion and following the autogamy. The initial cell valves were produced after at least two acytokinetic, pycnotic mitotic divisions, following which the cell was capable of undergoing its first normal vegetative division. SEM examination showed variability between species in the quantity of scale contribution to the auxospore wall.

DISTRIBUTION, GROWTH AND MICROZOOPLANKTON GRAZING OF FIVE PHYTOPLANKTON SPECIES IN LONG ISLAND SOUND INVESTIGATED BY REAL-TIME QUANTITATIVE PCR.

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Dilution method is a technique used in studying phytoplankton growth rates and mortality rates due to microzooplankton grazing. Numerous studies have been conducted utilizing this standard technique, using total chlorophyll as measurement of mean community growth and grazing rates. However, there are insufficient studies on the growth and grazing of specific phytoplankton species, although information on population dynamics and regulating mechanisms is often crucial for understanding the function of the ecosystem.
In this study we investigated distribution, growth and grazing rates of *Akashiwo sanguinea*, *Scripsiella* sp., *Cyclotella cryptica*, *Tetraselmis striata* and *Rhodomonas* sp. in Long Island Sound (LIS) representing four different classes of phytoplankton. Species-specific primers were developed based on the small subunit ribosomal DNA (18S rDNA) (*Cyclotella cryptica*, *Tetraselmis striata* and *Rhodomonas* sp.), mitochondrial cytochrome b gene (*cob*) (*Scripsiella* sp.), and PCNA gene (*Akashiwo sanguinea*). Samples were collected from seven stations in LIS from August 2002 through Septemper 2003, and abundance of the five species was quantified using Real-Time quantitative PCR. To further examine how the dynamics of these species was regulated, samples were collected from dilution experiments conducted during a Long Island Sound Integrated Coastal Observing System (LISICOS) cruise on March 2005. DNA was extracted and Real-Time quantitative PCR conducted to allow estimation of growth and grazing rates of these species. Relative importance of growth and grazing in regulating the population dynamics of these species will be discussed.

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GIRDLE BAND STRUCTURE IN THE FRAGILARIOID GENERA *STAUROSIRA* AND *STAUROSIRELLA* (BACILLARIOPHYCEAE).

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One of the shortcomings in the taxonomy of *Staurosira* Ehrenberg and *Staurosirella* Willliams et Round is the lack of sufficient characters to differentiate them at the morphological level. The tremendous variability and intergradations of some diagnostic features among several species make their separation difficult. One potential structure that may yield a number of distinguishing diagnostic features is the cingulum or girdle, which in fragilarioid diatoms is composed of a valvocopula that attaches directly to the valve interior and several additional siliceous belts each known as copula. In spite of the existence of a considerable amount of literature on fragilarioid diatoms, few investigators make reference to the structure of the girdle. Many protologues of new species give the impression that they simply follow the description of the cingulum of the corresponding genus with no extra effort devoted to the study of girdle bands in those particular species. The structure of the girdle has often been assumed to be a relatively simple structure with no variability within or across species. Therefore, entire genera are believed to include species with open and closed cingulum, with and without fimbriae, etc. More recently it has been shown that species within *Staurosirella* vary in the open/closed nature of their cingula. Evidently, more studies are needed to assess patterns of variability in girdle band structure of the different fragilarioid genera. Some preliminary details of the structure of the girdle in one species within *Staurosira* and another in the genus *Staurosirella* are presented. Detailed observations of the cingulum of *Staurosira*

construens Ehrenberg reveal a cingulum composed of closed and open girdle elements, which may represent the first report of this kind for fragilarioid diatoms.

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THE INFLUENCE OF COASTAL TOPOGRAPHY AND CIRCULATION PATTERNS ON POPULATION GENETIC STRUCTURE OF *FUCUS VESICULOSUS* L. IN THE GULF OF MAINE.

Jessica F. **Muhlin** and Susan H. Brawley. School of Marine Sciences, University of Maine, Orono, Maine 04469, USA.

Water motion influences gamete release in the brown alga *Fucus vesiculosus,* with release confined to calm, sunny conditions. Levels of water motion on opposite sides of a coastal point can vary greatly during the reproductive season. Consequently, environmental asynchrony across a point may cause temporal reproductive isolation. This isolation may result in genetic differentiation that is based on topography. Genetic differentiation may also result from spatial isolation (isolation by distance) of individuals that can reproduce with each other. We are testing whether populations of *F. vesiculosus* on opposite sides of a coastal point are genetically distinct. Six microsatellite loci were used to define genetic structure at a large spatial scale by comparing fucoids at Schoodic and Pemaquid Points (80 km apart) and on a smaller scale by comparing sites on either side of each point (separated by 100s of meters). Preliminary results indicate populations of *Fucus vesiculosus* from Schoodic Point are not differentiated from populations at Pemaquid Point. At the scale of opposite sides of a coastal point, population structure is not related to topography. Other factors, such as local currents, may influence genetic structure of *F. vesiculosus* along a coastal promontory. Fine-scale circulation studies using oranges as drifters demonstrate the potential for storm-detached, reproductive fronds of *F. vesiculosus* to raft around a coastal point as well as to disperse over large distances along the Maine coast. (Supported by NSF OCE-99043)

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A CELLULOSE SYNTHASE-LIKE (SUBFAMILY D) (*CSLD*) GENE FROM *COLEOCHAETE SCUTATA.*

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Cellulose is an important component of many plant and algal cell walls and exists as crystalline microfibrils composed of β-1,4-glucan chains. Cellulose synthase genes (*CesA*s) encode the catalytic subunits of the cellulose synthase enzyme in bacteria, algae, and land plants. *Cellulose Synthase-Like* genes (*CSL*s) are currently only found in land plants. It is hypothesized that the CSLD proteins, which are similar in amino acid sequence to CesAs, are involved in normal growth of root hairs and pollen tubes in land plants. However, their specific function is unknown. In Arabidopsis, one of the six known CSLDs, *AtCSLD3,* is involved in root hair growth. When this gene was mutated, the results indicated a distortion and rupturing of tip-growing cells. In tobacco, CSLD proteins are expressed in pollen tubes, which have a cellulosic cell wall. These tobacco pollen tubes do not express *CesA*s, indicating that CSLDs may function to produce cellulose in the absence of CesAs. In either case it is evident that the tip-growing cells in both plants have localized expression of the *CSLD*s. The first algal *CSLD* gene has been identified in the green alga *Coleochaete scutata* using degenerate primers and PCR techniques. Southern Blot analysis shows a 5 kb band from the digestion with HIND III. Further investigation using inverse PCR and *in situ* RTPCR will be done to further characterize the *CSLD* gene in *C. scutata*. Localization of

the gene will test the hypothesis that the hair tip cells on this alga express *CSLD*s. This project was supported by the National Research Initiative of the USDA Cooperative State Research, Education and Extension Service, grant number # (2003-35304-13233) and the University of Rhode Island Foundation.

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PIT PLUGS AND PLASMODESMATA IN THE ULVOPHYCEAE (CHLOROPHYTA): OCCURRENCE AND PHYLOGENY

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Few of the green algae that have been placed in the Ulvophyceae have perforated septa; cross walls between cells are either intact or (as in the Dasycladales and Caulerpales) lacking. Plasmodesmata, common among members of Chlorophyceae and the "charophyte/streptophyte" algae, have been found among ulvophytes only in the Trentepohliales and in one marine microfilamentous species from Chile, assigned to the genus *Sporocladopsis*. Plugged septa, resembling the pit connections of florideophyte red algae, appear in two species, *Smithsoniella earleae* and *Ctenocladus circinnatus*, the phylogenetic positions of which have not been established. We have found plasmodesmata in *Sporocladopsis* isolates from Australia and California, and plugged septa in two species from the Atlantic Ocean, *Acroblaste reinschii* Wille and *Huberiella perforans* (Huber) comb. nov. ined. (*Endoderma perforans* Huber, *Epicladia perforans* (Huber) Nielsen; *Huberiella* gen. nov. ined.). Perforate septa have evolved multiple times in the Ulvophyceae. *Sporocladopsis* is a member of the Ulvellaceae, not the Trentepohliales, in molecular sequence phylogenies. *Smithsoniella* is likewise a (basal) member of the Ulvellaceae, while *Acroblaste*, *Ctenocladus*, and *Huberiella* form a separate clade (Ctenocladaceae) sister to the *Bolbocoleon*, *Phaeophila*, and Kornmanniaceae clades at the base of the Ulvales.

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RECOVERY PATTERNS IN THE ROCKY INTERTIDAL ZONE OF FRENCHMAN BAY, MAINE, FOLLOWING ICE SCOUR: IMMORTAL *FUCUS*?

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Ice scour along the coast of Maine occurs regularly, but the severity of this disturbance and its effect on intertidal communities varies both seasonally and annually. Severe ice scour can cause the complete removal of intertidal organisms. In the winter of 2004, severe ice scour occurred in the upper and mid-intertidal zones along the northern shore of Frenchman Bay at Schoodic Point (Acadia National Park), Maine. We are studying community recovery and comparing it to previous studies of ice scour in the northwestern Atlantic. A particular focus is to determine the rate of recovery and whether fucoid canopy is reestablished by new colonization or regeneration from original holdfasts. Two sites, separated by 100s of meters, were monitored to define recovery patterns over a small spatial and temporal scale. Both sites contained a full canopy of *Fucus vesiculosus* (100% surface cover) in the mid-intertidal zone prior to the ice scour. Quadrats (0.25 m^2) were randomly positioned in the zones at each of the two sites. Point counts (100 points/quadrat) monitored changes in species diversity and abundance. The intertidal zone in April, 2004, contained substantial bare space and newly recruited barnacles; mature *F. vesiculosus* was found only in crevices, but many basal holdfasts remained on the more exposed, unprotected areas. Quantitative sampling began in July (2004); the first analysis in the mid-intertidal zone (n = 6 quadrats) showed 8 ± 6 % (mean ± SE) *F. vesiculosus* surface cover, 21 ± 11 % *Semibalanus balanoides* and 25 ± 7% bare

substratum. Many *F. vesiculosus* holdfasts were regenerating in July as clusters of upright thalli; the longest thallus per clump was 4.67 ± 0.36 cm (mean ± SE). Subsequent analyses revealed little change in species diversity and abundance or of the length of regenerating *F. vesiculosus* thalli. Size structure of *S. balanoides*, though, changed markedly as young barnacles grew in size and no longer could be differentiated from mature, previously larger, barnacles. Recovery of the fucoid canopy is occurring, but it cannot be determined whether new colonization or regeneration from holdfasts plays a greater role in the rate of this recovery. (Supported by OCE-99047 to SHB.)

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SOME BIOLOGICAL AND CHEMICAL CONSEQUENCES OF AMD, SEWAGE TREATMENT, AND THERMAL POLLUTION BELOW THE CONFLUENCE OF THE WEST AND NORTH BRANCHES OF THE SUSQUEHANNA RIVER AT BYERS ISLAND, NORTHUMBERLAND CO, PA.

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Throughout the fall of 2004, we examined the effects of acid-mine drainage, sewage effluent, and a coal-fired power plant on the ecology of the Susquehanna River in Northumberland County, Pennsylvania. The river is virtually divided into west and east channels by the presence of Byers Island in the middle of the river just south of the confluence of the north and west branches. A coal-fired power plant is located on the west channel while Shamokin Creek, an AMD impacted stream, flows into the east channel. We collected water samples for standard physical (temperature, pH, ORP, and TDS), and chemical (phosphate, nitrate, ammonium, and hardness) parameters. In addition, we deployed diatometers and prepared logs to serve as substrates from which to collect diatoms at three week intervals. Our results document the chemical and biological influence and persistence of the AMD plume from Shamokin Creek into the Susquehanna River for 5 kilometers from the southern end of Sunbury, PA. Furthermore, we document chemical and biological impacts of the thermal plume from the power plant on the west shore. Our data suggest that the waters of the west and the north branches of the Susquehanna River show little lateral mixing below the confluence even when impeded by an inflatable dam at Sunbury. Also, the chemical and physical plume from Shamokin Creek and the thermal plume from the coal-fired power plant together with the waters of the north and west branches make four chemically distinct streams within the river at Byers Island.

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HOSTS AND ASSOCIATES: A PHYLOGENETIC STUDY OF EPIZOOTIC *SAPROLEGNIA* SPECIES (OOMYCOTA, CHROMISTA)

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Twenty-eight single-spore isolates belonging to the order Saprolegniales (Oomycota, Chromista) were established from living or dead invertebrate or vertebrate hosts collected in the southeastern U.S. Partial 28S rRNA and complete rRNA internal transcribed spacer (i.e., ITS1, 5.8S, and ITS2) sequences were determined for these isolates, combined, and added to a data set including 72 other saprolegnialean isolates. Phylogenetic analyses of these data were used to study host-associate relationships and test the hypothesis that all watermold species are opportunistic epizootics. Putative epizootic watermolds belonging to five saprolegnialean genera (*Achlya*, *Isoachlya*, *Leptolegnia*, *Pythium*, and *Saprolegnia*) were identified on the basis of morphology and/or DNA sequence comparisons. Results imply that epizootic isolates are generally more closely related to one another than they are to clades containing

isolates that have yet to be recovered from animal hosts. This pattern is particularly evident among isolates assigned to the genus *Saprolegnia* and, to a lesser extent, in *Achlya*. Although the ability to infect animal hosts has almost certainly arisen and been lost independently in many watermold taxa, available data do not support the hypothesis that extant species are all potential animal associates. These results also highlight problems with the concept of species and problems of identification that plague watermold systematics.

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MANAGING TRADITIONAL INFORMATION TO COMPLEMENT MOLECULAR DESCRIPTIONS OF THE ALGAL WORLD.

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The progressive transformation of biodiversity aspects of phycology to a molecular discipline contributes to the 'taxonomic impediment'. New technologies enhance species discovery and the resolution of inter-relationships, but other taxonomic activities – such as custodial responsibility for biodiversity information - are eroded. This erosion has the potential to isolate new observations from their general biological or ecological context. If that happens, the full value of new insights will not be realized, and work may be duplicated. This creates a challenge to mobilize traditional information so that it is e-accessible, and integrated with molecular insights. A layered approach to this problem has a number of advantages. This approach seeks to separate the factual or objective components of our knowledge and place them as a basal layer in an electronic knowledge world. This separation allows objective elements to be used in many different contexts and for each combination to be enriched with interpretative expertise. The creates new higher-order aggregates of knowledge that suit an individual and a context. The separation of objective knowledge from more contextualized knowledge is efficient because it allows objective knowledge to be acquired collectively and be placed in a communal resource. This removes duplication. Open access promotes high fidelity of factual information. The basal elements include knowledge domains such as names, ancillary nomenclatural information, descriptions (as historical statements), images, bibliography. Names are seen as the only available system of universal metadata for biology – capable of annotating all biocentric information. When names are packaged in a communal, editable and flexible classification (CU*STAR), they offer a vision of a cross-project indexing system capable of unifying biological knowledge on the internet. Basal or factual elements can be combined into 'dynamic fact sheets', and these incorporated with higher orders of organization (examples being micro*scope, Plankton*net, and Microbial Life (educational resources).

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A MORPHOLOGICAL EXAMINATION OF THE FRESHWATER DIATOM GENUS *FRUSTULIA* (BACILLARIOPHYCEAE) FROM COASTAL PONDS OF NORTH CAROLINA, USA.

Jeffrey M. **Pelczar** and Peter A. Siver. Department of Botany, Connecticut College, New London, Connecticut, 06320, USA.

The genus *Frustulia* was found to be an abundant and important component of the attached algal community in three suites of waterbodies along the coast of North Carolina: the Pocosin Wildlife Refuge, the Croatan National Forest and the Bladen Lakes region. *Frustulia*, along with the genus *Eunotia*, were found to account for over 50% of the number of valves from most of the study sites. This was not surprising given that the waterbodies were very acidic (most with a pH < 4), mostly humic-stained and relatively dilute. The objectives of this project were to 1) characterize the species present with light and

scanning electron microscopy; 2) examine the importance of different morphological characters and; 3) compare our findings with results from a similar survey from the Ocala National Forest in north-central Florida. Based on our preliminary findings, at least nine species were found in the study, including *Frustulia krammeri*, *F. saxonica*, *F. crassinervia*, *F. pseudomagaliesmontana*, *F. bahlsii*, *F. septentrionalis*, *F. pangaeopsis* and two possibly undescribed taxa. In addition to valve shape and size, striae density and areolae density, the structure of the helictoglossae, raphe fissures and areolae were all found to be useful and important characters in distinguishing between species. As reported in our previous work, we observed three distinct types of helictoglossae, referred to as linear, rolled-tongue and hoop-shaped, which correlate well with other sets of morphological characters. The diversity of forms in the North Carolina sites surpassed that described for the Ocala region.

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THE EFFECT OF IRON CHEMISTRY ON PHYTOPLANKTON COMMUNITY STRUCTURE AND CARBON CYCLING IN THE OCEANS.

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Large-scale iron fertilization experiments have shown that in some regions of the world's oceans phytoplankton growth is limited by iron. Iron addition to these waters generates a rapid response of large, fast growing diatoms, however, in some cases these diatom blooms do not fully form and instead the community shifts to smaller phytoplankton. What might cause such a change in the phytoplankton community shortly after iron fertilization? It may be linked to changes in the iron chemistry. Iron is added in a free, dissolved form that is available to diatoms, however the iron quickly becomes bound to naturally occurring organic ligands. It appears the diatoms cannot readily obtain iron from these chemical species and thus re-experience iron limitation in the midst of a surplus of iron. The source and chemistry of these natural ligands is largely unknown, however, they appear to play an important role in determining ecosystem structure and ultimately, carbon cycling in the ocean.
The primary objectives of my thesis research are to ascertain the effects of ligand-bound iron on phytoplankton productivity, species composition and carbon export from surface to deep waters. The ability of various phytoplankton cultures to scavenge free iron over a wide range of concentrations is being investigated through iron manipulation experiments. Some phytoplankton are able to grow under exceedingly low iron conditions ($10^{-22.5}$M), while others cannot. Manipulation experiments have also been conducted in the field with natural phytoplankton populations using a continuous culture incubator. In these experiments, synthetic ligands believed to mimic natural ones, were tested to determine which phytoplankton were able to use ligand-bound iron. Results from these experiments will be put into the broader context of carbon export from surface waters and global climate change.

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EFFICIENT SINGLE-CELL ISOLATION OF PICOEUKARYOTES USING FLOW CYTOMETRIC CELL SORTING.

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Picoeukaryotic phytoplankton (1 – 3 μm) are ubiquitous in marine waters, but are poorly known. Due to limitations of traditional isolation techniques it has been challenging to study marine picoeukaryote diversity and dynamics. These picoeukaryotes are commonly observed using flow cytometry and are easily distinguished from cyanobacteria (*Synechococcus* and *Prochlorococcus*) and larger eukaryotes.

With automated flow cytometric cell sorting, viable picoeukaryote cells can be efficiently sorted to establish clonal cultures. These cultures will complement molecular data to determine the genetic diversity of a specific region. We used a DakoCytomation MoFlo cell sorter to sample the diversity of picoeukaryotes from the Gulf of Maine. The picoeukaryote fraction was sorted into media-containing multi-well plates. Using different media types and plate formats (96-24 well plates) many clonal isolates from the picoeukaryote fraction were obtained from single cell and 10 cell sorts. Estimates of the sort success rate using single cell sorts of cultured picoeukaryotes were determined to be between 1 and 5%. Due to the high concentrations of picoeukaryotes in marine surface waters, and the speed with which the cell sorter can perform single-cell sorts, it's possible to efficiently obtain clonal isolates from field samples. Two samples from the Gulf of Maine were obtained for isolation and diversity studies, one from Jordan's Basin in the central Gulf and one from a coastal site. With both samples the picoeukaryote fraction was sorted, incubated, monitored, and clonal cultures isolated (one cell sorts). Many different picoeukaryotes were obtained based on differences in morphology and growth characteristics. Flow cytometric sorting provides an efficient method to sample the microbial diversity and obtain novel cultures.

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CYTOPLASMIC CALCIUM OXALATE CRYSTALS IN *CALLIPSYGMA WILSONI* (BRYOPSIDALES, CHLOROPHYTA) AND IN SELECTED CLADOPHORALEAN MARINE GREEN ALGAE.

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Light microscopic study of the giant-celled, marine green alga *Callipsygma wilsonis* (Bryopsidales) revealed numerous birefringent crystalline inclusions in the terminal segments of the assimilatory axes. The inclusions were thin, wedge-shaped plates with a triangular shape in face view, a base up to 60 µm long, and a height that was one-seventh the length of the base. Chemical solubility tests showed that the crystals were composed of calcium oxalate. Calcium oxalate crystals previously were reported to occur in the large central vacuoles of several bryopsidalean species, but the crystals in *C. wilsonis* were present in the parietal cytoplasm and moved in streaming cytoplasm. Living cells of marine members of the Cladophorales were examined to determine the sites of localization of calcium oxalate crystals. Cytoplasmic streaming was not found in members of this group, but small calcium oxalate crystals in *Chaetomorpha coliformis*, *Apjohnia laetevirens* and *Valoniopsis pachynema* were closely associated with the thin layer of parietal cytoplasm. These findings add to a growing body of evidence that calcium oxalate crystals in diverse algae may originate in cytoplasmic vesicles and appear in the central vacuole only secondarily.

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GENETIC VARIATION IN *GAMBIERDISCUS TOXICUS*.

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Gambierdiscus toxicus Adachi et Fukuyo 1979 is an armored toxic marine dinoflagellate that is epiphytic on macroalgae, sand and detritus. Certain strains of *G. toxicus* produce toxins associated with a form of food poisoning called ciguatera; however, not all strains of *G. toxicus* are toxin-producing. Variation in toxin production is hypothesized to be genetically determined, which may explain why ecological patterns

of *G. toxicus* abundance are not always indicative of fish toxicity. However, a comprehensive phylogenetic characterization of globally distributed strains is still lacking, as is the identification of molecular markers useful for discriminating between toxic and non-toxic strains. The purpose of this research is to investigate phylogenetic patterns using globally distributed isolates of *G. toxicus* to determine if genetic strains are grouped according to geographic location and if toxin production is concordant with phylogeny. A preliminary phylogenetic characterization of several *G. toxicus* strains using LSU rRNA will be presented. A simple method for the single cell extraction of DNA from both live and preserved dinoflagellate cells will also be discussed. This study will complement an evaluation of naturally-occurring phenotypic plasticity of *G. toxicus* by comparing morphological variation using cells isolated from field samples collected from Johnston Atoll, Pacific Ocean, compared with intra and interclonal variations in laboratory cultures.

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MARINE PICOCYANOBACTERIA: GENOMIC INSIGHTS INTO ECOTYPIC DIVERSITY.

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The unicellular marine cyanobacterium *Prochlorococcus* comprises a dominant portion of photosynthetic biomass in the open oceans. The genus *Prochlorococcus* consists of two physiologically distinct ecotypes that are suited for optimal growth in either surface or deep waters of the oceanic euphotic zone and consequently have different vertical distributions in the ocean. Analyses of the complete genome sequences of three strains of *Prochlorococcus*, one of the high light adapted ecotype (MED4) and two of the low light adapted ecotype (SS120 and MIT9313) have provided insight into the selective pressures these cells face in the open ocean. The three strains differ in their 16S rRNA sequence by less than 3%, yet are dramatically different on a whole genome level. The MED4 strain has a genome size of 1,657,995 bp with a %GC content of 30.8%, while the SS120 genome is 1,751,080 bp with a %GC content of 36.4%. In contrast the MIT9313 genome contains 2,410,873 bp and has a %GC content of 50.7%. The comparative architectures reveal dynamic genomes which have been shaped by genomic rearrangements, deletions and insertions. Although the three strains share a core set of genes, a significant number are not shared, which have either been differentially retained from the common ancestor, or acquired through duplication or lateral transfer. Some of these genes play obvious roles in determining the relative fitness of the ecotypes in response to light, nitrogen, phosphorus and iron availability, and hence in regulating their distribution and abundance in the oceans. Recent shotgun sequence assemblies recovered from *Prochlorococcus* in the Sargasso Sea suggest that there is a high degree of microdiversity at the genomic level within a single ecotype. More extensive analyses of this metagenomic data may reveal loci actively under selection in the marine environment.

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MOLECULAR CLONING OF GENES DIFFERENTIALLY EXPRESSED IN THE SILICIFIED CULTURE OF *SYNURA PETERSENII*.

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The Synurophyceae is a class of golden-brown, freshwater algae characterized by highly organized siliceous scales surrounding the cells. *Synura* petersenii is one of the most widespread species, and we chose it to be our model organism. In a culture medium lacking silica, *Synura petersenii* cells stops producing scales, even though cells continue to grow. The scale-forming process is induced again when silica is again added to the medium. At present, the genes regulating the silicification process are

unknown. The main objective of this study is to identify and sequence genes that are specifically involved in scale formation. To identify only the silicifying genes, we are using the suppression subtractive hybridization (SSH) method. This technique delineates the differential gene expression between silica scale-producing culture (S+) and nonscale-producing culture (S-). Forward and reverse subtractions were performed to identify candidate genes differentially expressed between S+ and S-. A total of 68 differential expressed plasmid clones were sequenced, and each was analyzed by BLAST programs. These clones will be subjected to differential screening by a dot blot cDNA array. The *in vivo* functions of the genes remain further investigation.

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INVESTIGATING THE MECHANISM OF CHEMOACCUMULATION IN *ASTREPHOMENE GUBERNACULIFERA*.

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A. gubernaculifera is a colonial green alga of the class Volvocales. Although there are no cytosolic connections between the cells of the colony, it swims as a unit, and can accumulate in response to light and/or chemicals. Thus, this system is one of the simplest possible examples of organismal behavior. Our lab has shown that *A. gubernaculifera* chemoaccumulates to acetate or propionate. The goal of our study is to gain insight into the mechanism by which the colonies accumulate. Several possible models require the alga to change speed or rate of turning in response to increased acetate. We used a video microscope attached to a VCR to record the movements of alga colonies from 15 to 30 seconds after placing cells from an acetate free solution into a homogenous solution of 10 mM acetate (experimental) or acetate-free solution (control). The coordinates of all colonies in the field were recorded and analyzed to determine the average translational and rotational velocities of each colony for that time period. We conducted four trials of algae exposed to acetate and four control trials. The average linear velocity of the acetate-exposed colonies (average =48.4 μm/s, standard deviation =47.2 μm/s, n=170) and those not exposed to acetate (average =43.9 μm/s, standard deviation =43.9 μm/s, n=147) were not significantly different using the Student's T-test. The rotational velocity of the colonies exposed to acetate (average = 114.58 degrees/s, standard deviation=30.22 degrees/s, n=170) and those without acetate (average = 112.76 degrees/s standard deviation=28.187 degrees/s, n=147) were also not significantly different according to a Student's T-test. It is possible that the behavioral changes were over by the time we were able to initiate tracking. We are presently quantifying swimming behavior of colonies as they enter into or exit from areas with added acetate.

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MITOCHONDRIAL GENOME DIVERSITY IN EUGLENOZOA

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Euglenozoa, composed of Kinetoplastida, Euglenida and Diplonemida, are a highly diverse group of flagellated protists, many of which have been studied in great detail morphologically. While the monophyly of Euglenozoa is now well supported, the phylogenetic relationship among the three Euglenozoan groups is still controversial. Kinetoplastida have been particularly well investigated regarding their mitochondrial genome (kinetoplast), the structure of which consists of a concatenated network of maxi- and mini-circles, and the genes it encodes undergo post-transcriptional modification

(RNA-editing). There are sporadic evidences that Diplonemida and Euglenida, both poorly investigated at the molecular level, might share similar characteristics. For instance, ultrastructure analysis of *Petalomonas cantuscygni*, a Euglenida considered to be one of the earliest branching taxon in this group suggests kinetoplast-like structures. Moreover, we showed recently that the mitochondrial genome of the Diplonemida *Diplonema papillatum*, is composed of multiple small circular chromosomes. Our aim is to survey in a taxonomically broad fashion mitochondrial genome architecture and expression in diplonemids and euglenids. By doing so, we hope to obtain a better appreciation not only of the mitochondrial genome diversity but also of the phylogenetic relationships within these groups. We will present preliminary data of (i) the complexity of mitochondrial DNA of *Rhynchopus* sp1., and (ii) on newly developed purification and culturing strategies of the Euglenida *Petalomonas cantuscygni* and *Peranema trichophorum*.

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AN IMPROVED METHOD FOR DETERMINING PHYCOBILIN PIGMENT CONTENT FROM CRUDE AQUEOUS EXTRACTS OF *PORPHYRA* (BANGIALES, RHODOPHYTA).

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There is a need in aquaculture, ecological and physiological studies for a fast, easy and reliable method of quantifying phycobilin pigment content in red seaweeds. Several protocols have been published for determining phycoerythrin (R-PE) and phycocyanin (R-PC) content from crude aqueous extracts. One frequently cited method utilizes peaks and troughs of absorbance spectra. Troughs absorbance values are used to establish a linear or logarithmic baseline attributable to background scatter of particulate cellular debris not removed by centrifugation. Pigment contents are calculated by subtracting corresponding baseline values from R-PE and R-PC absorbance peaks. The baseline correction is intended to make the method independent of centrifugation time and/or speed. However, when crude extracts of *Porphyra* were analyzed using this method, R-PE and R-PC estimates were significantly affected by centrifugation time, suggesting that the method may not be reliable for the genus. We have re-examined this and other existing methods and have developed an improved protocol for determining phycobilin contents of *Porphyra*.
We found that with sufficient centrifugation, background scatter in *Porphyra* extracts can be removed; the remaining spectrum reflects the overlapping absorbance peaks of water soluble pigments in the extract. Using fourth derivative analysis of extracts from *Porphyra* samples, we identified peaks corresponding to Chl *a,* R-PE, R-PC, and allophycocyanin (APC). Dilute solutions of purified R-PE, R-PC and Chl *a* were scanned separately to determine spectral overlap. From these scans and published extinction coefficients, equations were developed to calculate R-PE and R-PC concentration in mixtures of these solutions from absorbance measurements. The equations were then used to estimate R-PE and R-PC content in centrifuged *Porphyra* extracts. Purified R-PE, R-PC and Chl *a* solutions were mixed with concentrations corresponding to the sample estimates. Absorbance and fourth derivative spectra of the sample extract and purified pigment mixture were compared and found to closely coincide. The newly derived equations were found to be more reliable for *Porphyra* than equations in previously published methods.

THE PLASTID GENOME OF THE HAPTOPHYTE *EMILIANIA HUXLEYI*: AN EVOLUTIONARY STUDY.

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Haptophytes are unicellular, photosynthetic, flagellate eukaryotes, which play a key role in the global carbon cycle. Haptophytes, together with cryptophytes, heterokonts and dinoflagellates are the only lineages that contain secondary plastids with chlorophyll c as a main photosynthetic pigment. These four lineages have at times been grouped together on the basis of their pigmentation. However, the phylogenetic relationships within these algae are still unknown. The haptophytes are the last major group of algae whose plastid genome has remained largely uncharacterized. Here, we report the complete sequence of the chloroplast genome of the haptophyte *Emiliania huxleyi* (Lohmann) Hay et Mohler 1967 (CCMP#373). This species belongs to the coccolithophorids, the main group in the Haptophyta, and is likely to be the most globally abundant haptophyte. The genome is circular and consists of 105,309 bp with two inverted repeats of 4,841 bp each. In terms of both genome size and gene content *E. huxleyi* cpDNA is substantially smaller than any other from the red plastid lineage. The genetic information is densely packed, with 86.8% of the genome specifying 110 identified protein-coding genes, 9 open reading frames, 28 different tRNAs, and 3 rRNAs. The sequence of the *E. huxleyi* plastid genome represents an important source of information that may help to elucidate the origin of the plastids in the chlorophyll *c* containing algae, and the number of endosymbiotic events that took place in their evolution. Comparisons with the plastid genomes of diverse photosynthetic eukaryotes, regarding gene content, gene clusters, gene function were made. These data suggest that the plastids of the chlorophyll *c* containing algae are rather closely related to each other. This is consistent with a single origin of these plastids from the red algae. In addition, we performed phylogenetic analyses based on concatenated datasets to understand the plastid relationships among the chlorophyll *c* containing algae.

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LIGHT AND NUTRIENT INFLUENCE ON PHYTOPLANKTON GROWTH IN HUMIC LAKES.

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The interaction of light and nutrients in regulating summer phytoplankton growth was tested in seven pristine, small and darkly-stained forest lakes. These lakes are unusually in having experienced almost no sport fishing in the past 70 years. The phytoplankton flora was extremely rich (369 taxa) and was dominated by chrysophyte flagellates (87 taxa) and coccoid green algae (148 taxa). In situ incubations of two-way factorial design- light (manipulated by incubation depth) X nutrients (natural vs enrichment with N, P) – surprisingly indicated that nutrient additions alone significantly increased algal growth rates in all twenty four experiments. Significant growth rate changes in response to light manipulations only occurred in the two most darkly-stained lakes, or in combination with nutrient enrichment. This pattern appeared related to the background available phosphorus concentrations in the lakes. Stoichiometric analyses of plankton particulate matter suggested that phosphorus was the likely limiting nutrient at the time of each experiment in each lake. Expression of experimental phytoplankton growth rates (140 data points) as functions of estimated cellular phosphorous quota and of integrated total light input during the incubation (PAR from solar radiometer, corrected for water column extinction) resulted in saturating functional relationships with positive X intercepts, as expected from Internal Stores Model kinetics. Combination of these relationships generated a three-dimensional model that closely describes light and

phosphorus requirements for positive growth and for maintaining maximum growth rates of phytoplankton in these seven brown-colored lakes.

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ISLAND BIOGEOGRAPHY AT A MICROSCALE: SPECIES-AREA RELATIONSHIPS OF DIATOM COMMUNITIES IN PENNS CREEK, SNYDER CO, PA.

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Our investigation is a continuation of a two year study to test a species area relationship between diatom periphyton and substrate surface area in Penns Creek, Snyder County, Pennsylvania. Past data on that area of Penns Creek showed a strong relationship between surface area of the stones (log area in cm^2) and the log of the number of diatom species present ($R^2 = 0.92$). This year, we deployed 50 ceramic tiles in five different size categories (16 cm^2, 49 cm^2, 100 cm^2, 225 cm^2, and 225 cm^2) in Penns Creek through the months of June and July at two different time intervals: six weeks and three weeks, respectively. Ceramic tiles were chosen to eliminate variation due to substrate composition and surface topography. During the six-week interval, we removed half of the tiles from the creek at three weeks. The diatoms harvested from these tiles were scraped and the frustules were acid-cleaned and prepared for examination under the light and scanning electron microscopes. The species-area regression lines ($R^2 = 0.89 – 0.97$) all had similar slopes. In addition, the species numbers ranged from 7-17 on the $16cm^2$ tiles up to 27-32 on the $225cm^2$ tiles. All tiles included *Cocconeis placentula, Diatoma vulgaris,* and *Navicula lanceolata* as their dominant taxa. Our results together with previous research confirm that tiles, like rocks, can be treated as islands, and that species diversity increases on larger "islands" as suggested by the theory of Island Biogeography.

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PROTOCOLS FOR CRYOPRESERVING MARINE PHYTOPLANKTON.

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Cryopreservation is the freezing and storage of living cells at very low temperatures (less than -135° C), and it offers several advantages. Cryopreserved strains reduce or remove serial transferring of actively growing strains, saving substantial work (e.g., culture medium preparation, washing and sterilization of glassware, perpetual subculturing of strains). Cryopreservation also reduces or eliminates potential contamination, possible mislabeling of strains and other errors that derive from repeated human handling. The "evolution" of strains during serial transfer is eliminated when strains are maintained at very low temperatures. Many strains of marine phytoplankton are easily cryopreserved using routine method, e.g., using cultures in log growth phase and a cooling rate of -1° C per minute in the presence of dimethyl sulfoxide (DMSO). DMSO (5-12%) is especially effective for chromalveolates whereas 5% methanol works for many green algae. In general, species with small cells cryopreserve more successfully than species with large cells. Long term storage of cells must be below -135° C, preferably lower than -160° C, in order to maintain an unchanging viability of the frozen cells. Typically, the cells are thawed very quickly in a water bath (35-40° C), the cryoprotectant is removed, and cells are innoculated into fresh culture medium. The CCMP has successfully cryopreserved over 1000 strains of algae and these will be summarized along with specific freezing protocols.

THE SILICA SECCHI DISK: AN INTERACTIVE PHYCOLOGICAL AND LIMNOLOGICAL TOOL.

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The Freshwater Ecology Laboratory at Connecticut College has developed an extensive interactive website, http://silicasecchidisk.conncoll.edu, that provides research tools for diatoms and scaled chrysophytes, an extensive limnological database, and educational aids for the study of freshwater algae. The latest addition to the Silica Secchi Disk site is an identification key to freshwater algal genera created with Lucid Professional software. The Key to Freshwater Algae allows users to quickly and efficiently narrow down a list of taxa to only those that match the characteristics they have chosen. This non-hierarchical, user-friendly key is linked to web pages containing a wealth of resources, including images, movies, a glossary, and information about the morphology, ecology, and reproduction of each organism. Our key and its supplemental materials provide an innovative alternative to traditional dichotomous keys that is particularly appropriate for introducing students to common algal genera. Visitors to the site may also search a full database of organismal and lake ecological data from our research in lakes and ponds in northern New England (Maine, Vermont, New Hampshire), the Adirondacks (New York), Cape Cod (Massachusetts), Connecticut, North Carolina, and Florida. Those interested in diatoms and scaled chrysophytes common to North America may access an image library of light and scanning electron micrographs and search the database for ecological information, biogeographic records, and taxonomic data. A complementary section highlights images and references for new chrysophyte and diatom taxa resulting from our research efforts. Interactive taxonomic keys are also available for some chrysophyte taxa. The Silica Secchi Disk site allows visitors to access a variety of phycological and limnological information generated by the Freshwater Ecology Laboratory as well as unique, interactive educational tools.

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HOW OLD ARE SCALED CHRYSOPHYTES? FOSSILS FROM THE GIRAFFE PIPE DEPOSIT, NORTHWEST TERRITORIES, CANADA.

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Fossils believed to be chrysophyte stomatocysts first appear in the geologic record during the Cretaceous, close to the time period when flowering plants evolve and begin to radiate. At this same time silicoflagellates and marine diatoms appear in the fossil record and there is a peak in the abundance of coccolithophorids. Despite the appearance of stomatocysts close to 120 million years ago, to our knowledge there are no records of siliceous scales representing either chrysophytes or synurophytes from the Cretaceous, nor from the subsequent Cenozoic Era. We found abundant remains of scales and bristles in two strata from the Giraffe Pipe deposit in Northwest Territories, Canada that are ca. 48 million years old. The Giraffe Pipe is a kimberlite deposit that contained a small, deep, closed-basin lake that over time filled and became a terrestrial site. The sedimentary strata were found between 50 and 163 meters in a drill core. The scales are well preserved and represent taxa from the genera *Mallomonas, Synura, Chrysospharella* and *Spiniferomonas* and include at least ten different species. Some of the taxa have not been reported in modern-day studies and presumably represent new and probably extinct species. Others are astonishingly similar to extant species and differ only slightly from their modern counterparts. Both simple as well as complex scale designs are represented. Based on our findings, the unique siliceous ornamentation of the scaled chrysophytes, including scales, spines, and bristles, was fully evolved by the

middle Eocene. These observations imply that the scaled chrysophytes experienced prolonged intervals of relative evolutionary stasis during Neogene and Quaternary times, and thus provide a new evolutionary milepost for this algal group.

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A PRELIMINARY INVESTIGATION OF THE *BATRACHOSPERMUM PSEUDOGELATINOSUM — B. CAMPYLOCLONUM* COMPLEX (BATRACHOSPERMALES, RHODOPHYTA) FROM AUSTRALIA AND NEW ZEALAND.

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Batrachospermum pseudogelatinosum and *B. campyloclonum* from Australia and New Zealand are broadly circumscribed species, being morphologically diverse, and are difficult to distinguish from each other. Specimens of these species were collected from locations in the provinces of New South Wales and Victoria, Australia, and in New Zealand including Stewart Island. DNA sequence data from a 1282 base-pair portion of the *rbc*L gene and the *cox*2-3 spacer region (~350 bp) were used to examine the phylogenetic relationships among these specimens and other closely related taxa. Preliminary results from the *rbc*L gene sequence data show two clades of specimens identified as *B. pseudogelatinosum*, with the well-defined species, *B. kraftii*, as sister to one of these clades. Maximum parsimony analysis of the *cox*2-3 spacer region data show a similar topology to the *rbc*L tree; three *B. pseudogelatinosum* specimens formed a well-supported clade in both analyses. Two specimens from the North and South Islands of New Zealand were initially identified as *B. campyloclonum* formed another well-supported clade. Biogeographic trends from the *cox*2-3 spacer analysis will be discussed.

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EFFECTS OF SIMULATED GLOBAL CHANGE ON ALGAE AND ASSOCIATED FOOD-WEBS.

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On-going environmental changes may be disrupting plant biochemical pathways and, through subsequent changes to nutritional quality (phenylpropanoids), associated food-webs/nutrient cycling. Whether algal species, or reliant consumer/detritivore communities, are similarly responding to long-term, multi-factor change remains unclear. Studies (days to months) exploring algal (Chlorophyta, Phaeophyta) responses (growth, biochemistry) to meso- and microcosm scale environmental manipulations (CO_2; UVR), as well as disruption to reliant freshwater (microcaddisfly) and marine (*Tegula funebralis*) herbivores and detrivores will be presented. Results indicate variable growth and nutritional quality responses among tested algal species, potential impairment of intertidal detritivores and subsequent nutrient cycling, but minimal disruption (feeding; development) to tested algivores.

THE GENETIC AFFINITIES OF *FUCUS COTTONII* WYNNE ET MAGNE FROM ROSMUC, IRELAND.

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The taxonomic status of the limicolous fucoid taxon *Fucus cottonii* Wynne et Magne has been the subject of much controversy. To provide insight into the origins of this unusual taxon, samples of four *Fucus* taxa from a salt marsh at Rosmuc, Ireland were collected and genotyped at four microsatellite-containing loci. The taxa included *F. cottonii*, *F. vesiculosus*, *F. spiralis*, and a morphotype of *F. vesiculosus*. The observed heterozygosity of *F. cottonii* at Rosmuc was much lower than that observed for muscoides-like populations within the northwest Atlantic. In addition, factorial correspondence analysis showed that *F. cottonii* displayed the greatest genetic similarity to *F. vesiculosus* and was not strongly affiliated with *F. spiralis*. By contrast, studies within the Gulf of Maine suggest that muscoides-like forms are often derived from hybridization between *F. vesiculosus* and *F. spiralis*; *F. cottonii* from Rosmuc is likely derived from *F. vesiculosus* and not of hybrid origin. Even so, genetic differences between *F. cottonii* and *F. vesiculosus* appear to be present and the designation of the former taxon as an ecad of the latter may be inappropriate. A morphotype of *F. vesiculosus* previously examined by Dr. Robert Wilkes was not genetically differentiated from individuals of *F. vesiculosus* displaying typical morphologies at the four loci examined.

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BIODIVERSITY OF BENTHIC ALGAL COMMUNITIES IN STREAMS AND THEIR PREDICTED ECOSYSTEM SERVICES.

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An emerging idea in ecosystem theory is that communities with greater species diversity or species richness sustain a higher level of ecosystem services. The addition of more species is predicted to increase the number and breadth of ecological niches to a community, which in turn provides greater functional roles. Because algae play important and diverse roles in the functioning of aquatic ecosystems, we propose that algal biodiversity should be examined from this same point of view. For example, "Community A" containing species of heterocystous cyanobacteria, such as *Rivularia* or *Calothrix*, may buffer inorganic nitrogen dynamics in a stream, while "Community B" lacking species with these physiological traits may exhibit less stable N cycling patterns. A network of many algal species should therefore offer greater nutritional diversity for consumers and thus greater food web stability. We have begun intensive survey of the biodiversity of benthic and transported algae in streams across New York State. A key objective is to assess the link between algal species richness and a suite of properties we define as ecosystem services provided by algal assemblages. This paper presents the experimental design and conceptual basis for our survey. We will address biodiversity from five perspectives: (1, 2) genus and species richness, (3, 4) genus and species diversity, and (5) chlorophyll + carotenoid pigment diversity. From the sampled assemblages we will measure six attributes that we suggest offer ecosystem services, in the form of biomass measured as (1) carbon, (2) chlorophyll-*a*, and (3) ash-free biomass, plus the nutritional / physiological properties of (4) C:N:P stoichiometry, (5) phosphomonoesterase enzyme activity, and (6) concentrations of fatty acids. Our preliminary data indicates that species richness as well

as species identity affect the ecological stoichiometry (C:N:P ratios) and fatty acid composition of benthic algal communities, and that these properties should have important ecosystems consequences.

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LOCALIZATION OF ENDOSYMBIOTIC BACTERIA IN *CAULERPA CUPRESSOIDES* WITH EMPHASIS ON ALPHA-PROTEOBACTERIA AND RHODOBACTER.

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The presence of endosymbiotic bacteria in *Caulerpa cupressoides* has been described in the scientific literature. Previous work in our lab has identified Alpha-proteobacteria and Rhodobacter as part of this community. However, the relative abundance and localization of these endosymbionts has yet to be described. This study utilized fluorescent staining techniques to detect bacteria within fixed cross and whole mount sections of the apical tips, stipes, blades, and rhizoids from *C. cupressoides*. A general estimation of the bacterial population was made using DAPI (4',6-diamidino-2phenylindole) stain which labels DNA. Two more specific probes, one for Eubacteria and the other specific for Rhodobacter, were hybridized to the bacterial symbionts within *C. cupressoides* using fluorescent in situ hybridization (FISH). An epifluorescent microscope capable of taking serial digital pictures with Z stacking and 3D reconstruction was used to visualize the endosymbiotic bacteria. Early results suggest that the Eubacteria and Rhodobacter represent a relatively small part of the bacterial population. Based on electron micrographs it appears that the majority of the bacteria reside in the vacuole. Many bacteria appear to be grouped near the cell wall trabeculae that extend throughout the cytoplasm.

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AN HISTORICAL ACCOUNT OF THE DEPICTION OF MARINE ALGAE: A PREVIEW.

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This project, culminating in a book manuscript entitled "Portraits of marine algae: an historical perspective," was undertaken to pay homage to the early workers who studied marine algae and depicted them with great attention to detail. The scope of this study covers from the 1760s to around 1900, selecting authors who include seaweeds in their treatments. The project includes 58 authors, 64 works (books or journal articles), and 86 plates and highlights authors who included seaweeds in their treatments. During this period covered, many voyages of exploration were bringing back collections of plants (including algae) and animals to the museums and universities of Europe, where they were studied and described. By default, it is mostly a "Euro-centric" exercise. Most of the authors (*e.g.*, W. Hudson, J. V. Lamouroux, R. K. Greville, J. G. Agardh, W. H. Harvey, and G. Zanardini) are reasonably well known to the phycological community, but others (*e.g.*, Velley, Bertolini, Delle Chiaje, and Corda) are less familiar. An effort was made to give a balanced representation of the three classes of macro-algae: the Chlorophyceae, the Phaeophyceae, and the Rhodophyceae. The layout of the presentation was to compile examples of the depiction of marine algae, to say something about the author, or authors, and to put their life and contributions in the context of their time. The following six early workers will be used to preview this project: S. G. Gmelin, F. X. Wulfen, C. A. Agardh, C. F. P. von Martius, C. Montagne, and J. D. Hooker.

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PHYLOGENETIC RELATIONSHIPS AMONG GLUTAMATE SYNTHASE ENZYMES.

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Nitrogen is incorporated into amino acids through the sequential reaction of two enzymes: glutamine synthetase (GS) and glutamate synthase (GOGAT). Although GS and GOGAT play a central role in regulation of nitrogen assimilation in photosynthetic eukaryotes, our knowledge of the evolution and regulation of these enzymes in non-green algae is limited. GOGAT isoenzymes catalyze the transfer of the amido nitrogen of glutamine to 2-oxoglutarate using pyridine nucleotides (NADH-/NADPH-dependent) or ferredoxin (Fd-dependent) as reductants. Fd-GOGAT is found strictly in cyanobacteria and photosynthetic eukaryotes. The gene is located in the chloroplast of rhodophytes and in the nucleus of vascular plants, but in both cases its product is active in the chloroplast. NADH-GOGAT is found in the nucleus of vascular plants, fungi, and diatoms, while NADPH-GOGAT is found in non-photosynthetic bacteria and archaea. In order to investigate the molecular evolution of GOGAT isoenzymes, we retrieved Fd- and NAD(P)H-GOGAT amino acid sequences from GenBank and the *Thalassiosira pseudonana* genome project. The N-terminus of the *T. pseudonana* Fd-GOGAT sequence is serine- and threonine-rich, indicative of a transit peptide targeting the gene product to the chloroplast. In equally-weighted maximum parsimony analysis, Fd-GOGAT sequences of vascular plants, chlorophytes, rhodophytes, and diatoms were found to stem from the cyanobacterial clade, with rhodophytes forming a sister group to vascular plants, while *Prochlorococcus marinus* and *T. pseudonana* were located more basally and appeared as sister taxa. NADH-GOGAT enzymes from fungi, vascular plants, and *T. pseudonana* formed another noteworthy group with fungi and vascular plants constituting sister clades, and with the enzymes from *T. pseudonana* and the oomycete *Phytophora* (each other's sister taxa) appearing to have diverged early. Our results are consistent with the hypothesis that the Fd-GOGAT gene in the diatom was transferred from the chloroplast genome of the endosymbiont to the nuclear genome of the host cell not long after the engulfment of the photosynthetic eukaryote by a non-photosynthetic eukaryote. In contrast, the NADH-GOGAT gene of diatoms may have been present in the host cell genome although sequences from rhodophytes are lacking. Further analysis of GOGAT evolution should provide insight into the history of endosymbiotic gene transfer events.

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A SURVEY OF THE DIATOM COMMUNITY IN A SMALL WOODLAND STREAM FROM SOUTHEASTERN OHIO.

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The majority of stream segments in southeastern Ohio have been impacted by acid mine drainage and it is difficult to assess the natural periphyton assemblages. This study provides baseline data for an unimpacted stream with an intact riparian corridor. The diatom community was sampled from erosional (riffles) and depositional (pools) habitats on a monthly basis. In addition, select water chemistry and stream physical attributes were measured. There was a total of 95 infrageneric taxa identified throughout the study. The samples from the depositional habitat, which tend to integrate the community over a long period and reflect upstream conditions, yielded a total of 85 species and the erosional, which reflect current stream conditions, showed 56 species. However, a large number, 46 taxa, were in common to the

two habitat types. A third of the taxa were rare, having <5 valves recorded throughout the study. There were four taxa that appeared to be dominant in terms of number of valves in a sample and present each sampling date. Those taxa are as follows: *Achnanthidium minutissimum, Synedra ulna, Cymbella delicatula* and *Encyonema minutum*. There was no significant difference in species diversity between the erosional and depositional habitats. In addition, PCO ordination did not show any seasonal patterns. This stream appears to be very stable in most water chemistry variables and diatom community composition. Species diversity and occurrence of individual taxa in this study and other research of unimpacted and impacted streams will be discussed.

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MITOCHONDRIAL CYTOCHROME *B* mRNA EDITING IN DINOFLAGELLATES: ASSOCIATION WITH EVOLUTION AND/OR RED-TIDE FORMATION?

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mRNA editing in dinoflagellate mitochondrial genes has been discovered recently. To gain further understanding on ecological and evolutionary associations of this intriguing phenomenon, we analyzed cytochrome *b* (*cob*) mRNA editing for six species representing distinct ecotypes and taxonomic classes of dinophyceae. We mapped the characteristics of *cob* editing to phylogenetic and ecological identities. Edited sites in all the six species aggregate in four clusters and occur predominantly at first and second positions of codons (93%), overwhelmingly involving A→G, U→C or C→U substitutions with smaller number of G→C, G→A. However, some other characteristics of editing vary between different taxa. Editing density (percentage of nt that are affected by editing) increases (2.15-4.08%) from early to derived lineages. Higher editing densities also map to red-tide forming lineages. Furthermore, comparison of location of edited codons and the type of nt changes in different lineages yielded identity matrices that mirror the taxonomic affinity of the species. Cladastic analyses based on the resultant distance matrices produced a cladogram that resembles the *cob* sequence-based phylogenetic tree. The results bolster our earlier hypothesis that *cob* editing is widespread in dinoflagellates and suggest that density, location, and type of editing-derived nt changes may bear yet-to-define evolutionary and/or ecological significance. Whether the association of high editing densities with red-tide species is a coincidence caused by association with the derived phylogenetic state of the species warrants rigorous examination.

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