J. Phycol. **47,** S1–S98 (2011)

DOI: 10.1111/j.1529-8817.2011.01050.x

ABSTRACTS

SYMPOSIA TALKS

THE EVOLUTION OF PROTISTS AND THEIR ORGANELLES: NEW INSIGHTS FROM THE FRONTIERS OF GENOMICS

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The availability of inexpensive genome and transcriptome sequencing capacity has furnished insights into the biology, biochemistry, evolutionary relationships and dynamics of protistan genomes at an unprecedented rate. From these data, large concatenated data sets of conserved protein genes have been assembled and phylogenomic analyses are converging to a stable picture of the inter-relationships of the major eukaryotic super-groups. At the same time, comparative assessments of gene contents of diverse microbial eukaryote genomes are allowing us to tease apart the relative impact of primary and secondary endosymbiotic organelle-based gene transfer versus lateral gene transfer in shaping the biochemical properties of these organisms and their subcellular compartments. In this presentation I will provide an example of how newly acquired genomic and transcriptomic data from non-photosynthetic protists have changed our understanding of: (1) the major supergroups of eukaryotes, (2) the true biochemical spectrum of mitochondrial function in free-living and parasitic protists and (3) the genome evolutionary mechanisms by which protists adapt to new environments.

INTEGRATED APPROACHES TO THE ECOLOGY OF WILD AND CULTURED PICOEUKARYOTES

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Half of global primary production is performed by photosynthetic marine organisms, including a diverse array of algae. Among these, algae are tiny unicellular eukaryotes referred to as ''picoeukaryotes''. Although picoeukaryotic taxa such as the prasinophyte *Micromonas* have been studied since the 1950's, others are still being discovered and many remain uncultured. Integrated genomic analyses that involve working with both cultured isolates and ''wild'' uncultured populations are a powerful way to investigate these important primary producers. Here, new approaches for studying marine picoeukaryotes and resulting insights on their evolution, diversity and physiology will be discussed.

ECOLOGICAL ASPECTS OF NITROGEN-FIXING CYANOBACTERIA ILLUMINATED BY GENOMICS AND METAGENOMICS

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Nitrogen is a key nutrient limiting the productivity of the oceans. Nitrogen fixation is an important source of nitrogen to the surface waters of oligotrophic oceans and was believed to be primarily due to the marine *cyanobacterium trichodesmium*, until the discovery of nitrogenase genes associated with other bacteria and cyanobacteria. The application of genomic and metagenomic approaches has unveiled unusual aspects of the metabolism of free-living and symbiotic cyanobacteria. Unicellular cyanobacteria lacking basic metabolic features such as photosystem II and the TCA cycle suggest that yet more symbioses are yet to be discovered, and these organisms appear to have a wider global distribution than other nitrogen-fixing cyanobacteria. Filamentous cyanobacteria in symbiosis also have unusual metabolic deletions. These findings, in concert with biogeographic distribution information suggest that nitrogen-fixation in the oceans by marine cyanobacteria is much more complex than previously believed.

COMPARATIVE GENOMICS OF MARINE DIATOMS

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About one-fifth of the photosynthesis on Earth is carried out by marine diatoms. Because of their important role in the global cycling of carbon, it is critical to understand how these organisms will respond to the changing conditions of the world's oceans. I will describe ways that comparative genomics can be used to develop new insights into both the evolution and ecology of diatoms. At least four whole genome sequences are currently (or soon will be) available for marine diatoms, with the number of available diatom EST sequences increasing rapidly. I will provide examples of new approaches to understanding genetic diversity and biogeography by comparing whole genome sequences derived from six strains of the model centric diatom, *Thalassiosira pseudonana* isolated from different oceans. I will also describe use of transcriptional analysis of both laboratory isolates and field populations to understand the response of diatom communities to environmental change.

THE *CHONDRUS CRISPUS* GENOME

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The red seaweeds represent the last large group of advanced multicellular organisms without a sequenced genome and this lack of knowledge has been a major obstacle in studies of red algal biology. We therefore decided to sequence the genome of the florideophyte *Chondrus crispus* (Irish moss), a common seaweed with a long research history found on rocky shores on both sides of the Northern Atlantic Ocean. The genome project is a collaboration between an international consortium and the French sequencing agency Genoscope. It is based on a 14-fold sequence coverage and transcriptional data of a gametophyte of *Chondrus*. A combination of automatic and expert annotation of the 105 Mbp genome has demonstrated approximately 9,500 protein coding genes, of which a large proportion have unknown functions. The genome is characterised by gene-dense regions surrounded by repeat-rich blocks. The genes are compact with only 0.2 introns per gene of limited size and short flanking untranslated regions. The genome is characterised by a low redundancy and small gene families, for example concerning ribosomal proteins and enzymes involved in floridean starch metabolism. Other notable features of the genome include a large number of genes involved in halogen metabolism, including 35 peroxidases and several dehalogenases. We anticipate that the sequencing and analysis of this genome as well as the creation of a large transcriptomic resource will establish *Chondrus crispus* as a relevant model organism for red macroalgae.

THE COMBINED EFFECTS OF OCEAN ACIDIFICATION, MIXING, AND RESPIRATION ON PH AND CARBONATE SATURATION IN AN URBANIZED ESTUARY

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In order to study the combined effects of ocean acidification and other natural and anthropogenic processes on Puget Sound waters, we made inorganic carbon measurements in this estuary on two survey cruises in February and August of 2008. Observed pH and aragonite saturation state values in surface and subsurface waters were substantially lower in parts of Puget Sound than would be expected from anthropogenic carbon dioxide (CO_2) uptake alone. We estimate that ocean acidification can account for 24–49% of the pH decrease in the deep waters of the Hood Canal sub-basin of Puget Sound relative to estimated pre-industrial values. The remaining change in pH between when seawater enters the sound and when it reaches this deep basin results from remineralization of organic matter due to natural or anthropogenically stimulated respiration processes within Puget Sound. Over time, however, the relative impact of ocean acidification could increase significantly, accounting for 49–82% of the pH decrease in subsurface waters for a doubling of atmospheric CO_2. These changes may have profound impacts on the Puget Sound ecosystem over the next several decades.

ACIDIFICATION AND WARMING IN THE RESPONSE OF MARINE PHYTOPLANKTON TO ENVIRONMENTAL CHANGE

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Additional atmospheric carbon dioxide increases the carbon dioxide concentration in the ocean, with an increase in dissolved carbon dioxide and bicarbonate and a decrease in carbonate and pH. These changes frequently decrease the calcium carbonate content of calcification of calcified phytoplankton, and either have no effect on, or sometimes commonly increase, the growth rate of phytoplankton under otherwise optimal conditions. Warming has direct effects on phytoplankton growth, and also causes shoaling of the thermocline with increased mean photosynthetically active radiation incident on cells and decreases phosphorus and combined nitrogen inputs to the upper mixed layer from the deeper ocean. Combined nitrogen input is also decreased by deoxygenated zones forming below the thermocline when there is a decrease in the downwelled oxygen flux relative to the sinking organic particle flux, increasing denitrification. These changes in nutrient and light availability increase photoplankton productivity at high latitudes but decrease productivity at lower latitudes, and decrease the effect of increasing carbon dioxide on increasing growth rate and repressing carbon dioxide concentrating mechanisms. More multifactorial growth experiments, and investigations of genetic adaptation, are required.

ASSESSING HUMAN ALTERATION OF PHYTOPLANKTON AND WATER QUALITY DYNAMICS IN A WORLD EXPERIENCING CLIMATIC CHANGE

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Harmful cyanobacterial blooms (CyanoHABs) are proliferating globally due to accelerating human nutrient enrichment. Traditionally, phosphorus (P) input reductions have been prescribed to control blooms because P-limitation is widespread and some CyanoHABs can fix nitrogen (N_2) to satisfy their nitrogen (N) requirements. However, anthropogenic N loading is increasing rapidly world-wide, promoting dominance by non N_2 fixing CyanoHABs that are N and P co-limited or N limited. Increasingly, N and P input constraints are needed for CyanoHAB control. Climatic changes, specifically warming, salinization, and intensification of storms and droughts, play additional, interactive roles in promoting CyanoHAB frequency, intensity, distribution and duration. Therefore, in addition to reducing in N and P inputs, water quality managers need tools to break the synergy between nutrient loading and climatic hydrologic regimes made more favorable for CyanoHABs by climate change. These include (1) altering the hydrology to enhance vertical mixing and/or flushing and (2) decreasing nutrient fluxes from organic-rich sediments. CyanoHAB management approaches must incorporate both N and P loading dynamics when considering altered thermal and hydrologic regimes associated with climate change.

WHO'S NEW IN THE NEIGHBORHOOD? CHANGING SEAWEED BIODIVERSITY AND ECOSYSTEM FUNCTION

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Seaweed biodiversity is changing in ecological time due to shifts in the distributions of native species and introductions of non-native species. After reviewing some of the evidence for changes in seaweed biodiversity and the ecological consequences, I will present results from a collaborative study (with Matt Bracken, Northeastern U.) on how natural biodiversity patterns in an intertidal seaweed community influence ecosystem functions of primary productivity and nitrogen acquisition. For many years, scientists have supported biodiversity conservation with evidence for a generally positive relationship between biodiversity and ecosystem function. Recently, ecosystem functions are being converted to ecosystem services of value to humans, to strengthen conservation and management efforts. This conversion carries both promise and risk, the balance between which depends strongly on the exact biodiversity-ecosystem function relationship and the processes that influence biodiversity.

MANAGING HUMAN IMPACTS ON ALGAL COMMUNITIES: PROSPECTS FOR THE FUTURE

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Human activities cause changes in algal communities through direct and indirect means. In the U.S., policy instruments exist to address some direct impacts to algal communities, but many direct impacts and most indirect impacts are without specific management or policy interventions. Employing more general approaches such as ecosystem management and spatial planning could offer means of reducing negative or undesirable impacts to algal communities, and linking ecological impacts to socioeconomic impacts could bring renewed attention to such problems. As human activities and their impacts on marine and freshwater systems persist and grow, new ways of addressing impacts to algae and their feedbacks to social-ecological systems will be required.

CIGUATOXINS AND BREVETOXINS

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Harmful algal blooms (HABs) (also known as ''red tides'') occur in all aquatic environments and are caused by algae which produce powerful natural toxins affecting humans and other animals. Ciguatera fish poisoning is caused by dinoflagellates of the genus *Gambierdiscus* which elaborate ciguatoxins; in coastal tropical areas throughout the world, persons consuming coral reef fish contaminated with ciguatoxins experience both severe acute and potentially chronic neurologic illness lasting weeks to months. *Karenia brevis,* the dinoflagellate associated with Florida red tides, elaborates brevetoxins; blooms of *K. brevis* cause significant fish, bird and marine mammal mortalities. In humans, brevetoxins are associated with neurotoxic shellfish poisoning (NSP), possibly neurotoxic fish poisoning, and respiratory irritation (especially in persons with underlying lung disease). As with all the HAB-associated diseases, the human illnesses caused by ciguatoxin and brevetoxin exposure are under-diagnosed and under-reported, yet may be increasing in incidence worldwide. Recent research involving exposure assessment, animal testing and human epidemiologic studies have increased our understanding of the potential exposures, the acute/chronic health effects of both these HAB diseases, and their potential mitigation and prevention.

CYANOTOXINS

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Algal toxins include some of the most potent natural chemicals known, and there is potential for human exposure in any community using surface water for drinking or recreation. Harmful algal blooms (HABs) occur when an overgrowth of algae threatens environmental, animal, or human health. Possible routes of exposure to HAB toxins include immersion, inhalation, and swallowing water or eating food containing the toxins. For marine HABs, there are established diseases and known adverse effects, including shellfish poisoning, ciguatera fish poisoning, and respiratory effects from aerosolized brevetoxins associated with Florida red tide events. However, we know much less about the health effects resulting from exposure to freshwater HAB toxins. CDC collects data on food- and water-borne disease outbreaks; however, these systems do not capture many recreational exposures or any individual cases of HAB-related illness. In response, the National Center for Environmental Health, in collaboration with partner organizations, created a unique surveillance system, the Harmful Algal Bloom-related Illness Surveillance System (HABISS), to capture human and animal health data as well as physical characteristics of HABs. My discussion today will focus on freshwater blue-green algae, or cyanobacteria, including potential exposure routes, possible health effects, and a summary of data from HABISS.

SAXITOXINS AND DOMOIC ACID: IMPACTS AND MANAGEMENT

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Seafood begins as sunlight, converted to biomass by algae. Seafood is generally wholesome and an important source of nutrition. Unfortunately, some algae produce potent toxins that can accumulate in seafood and cause illness and death in consumers. Paralytic shellfish poisoning is caused by the saxitoxins, a family of more than 20 related compounds, differing in potency but based on the same core structure. Saxitoxins are found in both dinoflagellates and cyanophytes. Once in animals, the saxitoxins act on several classes of voltage-activated sodium channel, binding reversibly at a receptor site that has no known function, blocking the transmission of impulses along nerve and muscle membranes. Blockage results in muscular and peripheral neural symptoms that vary with dose and resolve quickly as the saxitoxins wash out of the system. In severe cases, paralysis of thoracic muscles can lead to suffocation and death. With rare exceptions, respiratory support will assure survival. In contrast, domoic acid, responsible for amnesic shellfish poisoning in consumers, has several known isomers but appears to be the only compound in its family that has significant potency. Domoic acid is found in rhodophytes and in pennate diatoms. In mammals without excretory impairment domoic acid tends to cause gastrointestinal distress but little more. However, if it is not rapidly excreted, it may pass through the blood-brain barrier and bind to glutamate receptors, particularly those associated with the hippocampus, causing sustained stimulation and eventual cell death. Victims may suffer permanent mental impairment, particularly of short-term memory. In both cases, the primary strategy for avoiding human exposure is to monitor seafood stocks so that the harvest or consumption of toxic product can be avoided.

ALGAE IN THE KITCHEN (THE 2011 PSA PRESIDENT'S LECTURER)

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Ireland has a long history of seaweed use. This address will briefly look at some of the more traditional uses of seaweeds in Europe and move on to explore the nutritional properties of individual sea vegetables and current supporting evidence. The preventative and therapeutic roles of sea vegetables will be presented and these health benefits and current research, including ongoing work in the Northern Ireland Centre for Food and Health, will be discussed. How did land vegetables get so far ahead of their counterparts in the sea? An overview of the huge variety of uses of sea vegetables that can be enjoyed in everyday meals will hopefully encourage more researchers to focus on building the evidence and help with the challenge to get sea vegetables into mainstream use.

WORK OF THE PSA'S COMMITTEE ON NUTRITION AND FUNCTIONAL FOODS FROM ALGAE

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The Committee is currently reviewing published research on the nutritive value of algae as foods and experimental findings on potential functional benefits of consumption of algae as whole foods or as concentrates. A brief introduction to the goals of the Committee will be provided, including stimulation of the range of important pre-clinical and clinical studies needed to move this field forward.

NUTRITION FUNDING OPPORTUNITIES AND CHALLENGES

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A wealth of evidence points to the importance of diet in health promotion and disease prevention. Exciting funding opportunities exist at NIH, which currently provides ~ $1.5 billion annually to promote nutrition research across its institutes and centers. Numerous inconsistencies appear in the literature about bioactive food components as modifiers of cancer risk and tumor behavior. NSRG has been particularly interested in nutrigenetics, nutrigenomics, proteomics, metabolomics, and nutrient-nutrient interactions that may account for some of this variation. Double blind placebo control studies are the gold standard for this research stemming from solid preclinical evidence. A variety of funding mechanisms are available to scientists for this research including fellowships, grants and contracts. This presentation will review how to set the stage for competitive proposals in this area. Relevant examples about the potential role of algae as modifiers of cell proliferation, inflammation and immunocompetence related to cancer risks will be briefly discussed.

ENTERING THE FOOD SUPPLY WITH A NEW HEALTH-PROMOTING PRODUCT: REGULATORY AND NUTRITIONAL CONCERNS

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Nutrition and health, especially in relation to chronic disease, is a major driver of food product selection for many consumers. Entering the food stream with a product based on health-promoting properties requires successful negotiation of a number of scientific, regulatory and marketing hurdles. First the target market must be identified: either a dietary supplement, food additive or food. Next, the product must be thoroughly characterized (physical, chemical and toxicological profile) and a Good Manufacturing Practice protocol developed and validated. Ultimately regulatory approval must be obtained. Human health benefits may initially be investigated with in vitro and animal studies, but proof of efficacy for health requires human evidence, especially if label claims are to be made. All processes and procedures should be developed in conjunction with an efficient marketing program to avoid the high failure rate of new products, and this should help guide the decision regarding potential regulatory status.

MICRO-ALGAE FOR THE PRODUCTION OF BIOFUELS AND BIO-PRODUCTS

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Fuel, food, and biological products are all different forms of chemical energy, and as such are closely interrelated. All of these products are ultimately derived from photosynthesis, the process by which sunlight energy is converted to chemical energy. Over the last 100 years we have exploited fossil fuels to drive unprecedented economic and agricultural growth, but in so doing we have released sequestered CO_2 into the atmosphere, which is now beginning to impact our climate. In addition, fossil fuel reserves are finite, and we are now starting to see the initial signs of depletion of these reserves, including the rising cost of fuels and food. Together these factors have provided the impetus behind the development of new renewable energy sources that can supplant fossil fuels while greatly reducing carbon emissions into the atmosphere. Eukaryotic algae offer tremendous potential for the large scale production of biofuels and bio-products as algae require only sunlight as an energy source and sequester CO_2 during the production of biomass, and algae can be much more efficient then terrestrial plants in fixing CO_2 and producing biomass. We are developing the genetic tools to enable algae as a bio-fuels and bio-products platform, and have successfully introduced biosynthetic enzymes to modify hydrocarbon biosynthesis, as well as a variety of genes that allow production of valuable protein co-products in micro-algae. The challenges, potential, and some early successes of algae as a source of biofuels and bioproducts will be discussed.

CYANOBACTERIA AS SOLAR-POWERED BIOCATALYSTS FOR PRODUCTION OF BIOFUELS

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A unique feature of photosynthetic organisms is that they can use CO_2 and water as substrates, and sunlight as the energy source, to produce organic compounds that usually are applied for their own life processes. However, with fairly simple genetic engineering approaches genetically tractable cyanobacteria such as *Synechocystis* spp. PCC 6803 can be programmed to produce and excrete compounds that they cannot use themselves and that can be subsequently processed to biofuel. In this way, the organism serves as a biocatalyst and just the product needs to be harvested; biomass production is no longer a goal. This approach offers harvesting and processing advantages relative to a biomass-focused approach as cell harvesting, processing and extraction are laborious and expensive. Instead, our approach focuses on excretion of the biofuel product from the photosynthetic organism, so that just the product needs to be harvested. This also saves in nutrient inputs and optimizes recycling of resources. To produce and excrete the free fatty acid laurate, we equipped *Synechocystis* sp. PCC 6803 with a suitable thioesterase and deleted an enzyme used for the reutilization of free fatty acids. The resulting organism efficiently produces and excretes laurate, which subsequently is harvested. The harvested laurate is then converted to *n*-undecane by decarboxylation via the Centia™ process, and can be isomerized further to jet fuel, etc. Funded by ARPA-E.

FROM WATER OXIDATION TO STARCH, OILS OR HYDROGEN: PATHWAY ENGINEERING IN PHOTOTROPHIC MICROORGANISMS

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Photosynthetic microorganisms (PSMs) are able to synthesize a diverse portfolio of renewable energy carriers with the potential to alleviate the environmental and political concerns associated with fossil energy utilization. The reductant generated during photosynthesis is used to fix CO_2 into energy-rich organic molecules such as starches or lipids. Starch may be fermented into a host of bioenergy molecules including ethanol, butanol, H_2 and methane, and lipids may be converted into diesel fuel surrogates. Microalgae have high photosynthetic conversion efficiencies, are able to grow in a number of diverse environments, including salt water and industrial waste streams, and contain the enzymatic machinery for CO_2 fixation, anaerobic fermentation and H_2 production. This represents a distinct advantage over the majority of vascular plants, which do not possess this metabolic diversity and require limited freshwater resources for cultivation. Our research into directed enzyme evolution, metabolic engineering, and the dynamics of cellular transcript and protein accumulation is being used to develop a detailed understanding of the partitioning of photosynthetic reductant into the distinct metabolic pathways required for bioenergy accumulation in PSMs.

INSIGHTS FROM DIFFERENT APPROACHES FROM DESCRIPTIVE FIELD STUDIES TO MICROCOSM STUDIES

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The soil microbial loop concept is 30 years old. Yet our understanding of soil microbial food webs is still fragmentary. Four key research areas directly relevant to soil food webs are insufficiently taken into account: (1) Biodiversity: studied combining classical and molecular methods are revealing huge diversity both within known groups and novel unsuspected clades. (2) Biogeography: Not all free-living protists are cosmopolitan; what about functions? (3) Feeding habits and trophic position: What do all these species do for a living? How common are food-specialists? Can this information be integrated into models? (4) Spatial and temporal patterns: How can we account for complex spatial and temporal patterns when discussing soil microbial food web functioning? These important topics are typically studied by independent teams with different research backgrounds and scopes. New technological developments now allow assessing the full diversity of soil organisms and hopefully soon the position of each one in the food web. We now need to bring concepts and methods together to improve both theory and to design more ecologically realistic experiments.

HOW PROTOZOA STRUCTURE MICROBIAL COMMUNITIES IN THE RHIZOSPHERE OF PLANTS

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Protozoa are the primary consumers of bacteria in soil, but the consequences of protozoan predation for the composition and functioning of bacterial communities are poorly understood. Strong feeding preferences of protozoa paired with high consumption rates are rapidly shifting diversity, metabolism and function of bacterial communities in the plant rhizosphere, which has a profound influence on bacteria-plant interactions. Recent investigations indicate a complex chemical warfare between rhizosphere bacteria and protozoa. These interactions have been shown to alter plant physiology, gene expression, hormonal balances, the architecture of the root system, shoot development and plant biomass, and give evidence of the complexity of plant-microbe-protozoan interactions. Protozoa also interact with other plant symbionts, such as mycorrhizal fungi with profound effects on root growth and nutrient allocation. Because the regulation of root architecture is a key determinant of nutrient- and water-use efficiency in plants, our data show that microbial grazers significantly shape microbial interactions in the rhizosphere, and cannot be ignored if we want to achieve a mechanistic understanding of rhizosphere processes.

ESTIMATING N AND C TRANSFORMATIONS FROM STABLE ISOTOPE TRACER STUDIES IN AN INTACT SOIL FOOD WEB

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Protozoa are one of the most abundant groups of bacterivores within the soil, however they are regularly overlooked when investigating the complete soil food web. Little is known of their contribution to soil nutrient transfers or of the identity of their consumers. Within the soil decomposer food web there is little niche specialisation creating an ‘‘enigma of soil animal diversity’’. There are few methods that can be used to determine feeding preferences of the soil fauna in situ, but a technique using stable isotopes has been developed to track the feeding preferences as they occur. Here, for the first time indigenous flagellates and ciliates, were cultured to become highly enriched in ^{13}C and ^{15}N and were introduced to soil cores from two different land managements. This enabled the flow of protozoan C and N to be traced through the soil food web. We identified nematodes, Collembola, earthworms and insect larvae as dominant consumers of protozoa. Our results show the importance of protozoa as a food source and that changes in management, affect the functioning of the soil food web.

CONTRIBUTION OF PROTIST BACTERIVORY TO SOIL NUTRIENT CYCLING

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Soil protists and nematodes are the main consumers of bacteria in the soil. In contrast to the abundance of results on the ecology of the nematodes, there was scant data available on the contribution of protist bacterivory to soil nutrient cycling. Over the past 10 years we have broken enough ground with new techniques and statistical approaches to begin a synthesis and analysis of the overall protist contribution. This symposium brings together a variety of approaches used to obtain information on the various contributions of protist bacterivory, and to estimate the magnitude of this nutrient cycling. Abundances of active cells vary with climate, weather, soil organic matter content, soil types and structure. In addition, food web community structure, species composition and diversity, all vary with various ecological parameters. Results of functional response curves, prey preferences, and niche specifications indicate that as much as the standing biomass of bacteria are consumed daily by protists in temperate grassland soils. These results are being evaluated with new data using radioactive and stable isotope tracer studies. We are beginning to identify the main consumers of protists in the soil food web. This data allows us to understand community structure changes in soil food webs in response to succession (years) and longer term changes (decades).

BOLD TALKS

A FLORISTIC ANALYSIS OF THE SPATIAL VARIABILITY OF MARINE INTERTIDAL ALGAE BETWEEN CAPE MENDOCINO, CALIFORNIA AND CAPE BLANCO, OREGON

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Marine floristic information is critical to managers of coastal ecosystems and is useful in detecting changes in community structure over time. The biogeographic area between Cape Mendocino and Cape Blanco spans 320 km and lacks a current macroalgal species list. Therefore, this study aimed to (1) compare patterns of intertidal macroalgal species composition at four sites, and (2) compile a flora based on current and historical records. Collections were made during two field seasons and habitat attributes for each species were recorded. Cluster dendrograms based on a presence/absence matrix for each species assessed similarities in composition across sites. Comparisons were made with historical (Dawson and Doty) lists. A total of 162 species of macroalgae (103 Rhodophyta, 33 Heterokontophyta, Phaeophyceae, and 26 Chlorophyta) and two species of seagrasses were identified. The comprehensive list revealed a total of 322 species (201 Rhodophyta, 70 Heterokontophyta, Phaeophyceae, and 51 Chlorophyta) and four species of seagrasses indicating a relatively high area of biodiversity of 134 species per degree latitude. Further analysis is needed to evaluate whether this area is a biogeographic range barrier for macroalgae.

VARIATION IN MECHANICAL PROPERTIES AFFECTS HYDRODYNAMIC PERFORMANCE OF FOLIOSE RED SEAWEEDS

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Because hydrodynamic forces are likely the largest source of mortality for seaweeds in high wave energy environments, tissues with material properties that behave favorably in these environments are likely to be selected for. In this study, anatomical and material properties of 16 species of foliose red macroalgae were determined and their effects on hydrodynamic performance were measured in laboratory experiments holding size and shape constant. We found that increased blade thickness (primarily caused by the addition of medullary tissue) results in higher flexural stiffness which inhibits the seaweeds ability to reconfigure in flowing water, and thereby increases drag. However, this increase is concurrent with an increase in the force required to break tissue, possibly offsetting any risk of failure. Additionally, while increased non-pigmented medullary cells may pose a higher metabolic cost to the seaweed, decreased reconfiguration causes thicker tissues to expose more photosynthetic surface area incident to ambient light in flowing water, potentially ameliorating the metabolic cost of producing these cells. Material properties can result in differential performance of morphologically similar species.

LEVELS OF SELECTION AND ADAPTIVE HYPOTHESES FOR TOXIN PRODUCTION IN MICROALGAE

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Many microalgae invest in toxins that have profound impacts on coexisting species or entire communities. The taxonomic and biochemical diversity of algal toxins suggest a corresponding diversity of function in nature. Adaptive hypotheses range from non-adaptive byproducts, assistance in predation, anti-competitor allelopathy, and grazer deterrence or killing. These hypotheses differ in the levels at which the proposed benefits of toxin production are realized, from individual cells (e.g. a single cell benefits when a predator chooses nontoxic alternatives) to entire populations (a population benefits when indiscriminate grazers are impaired). Natural selection can act upon multiple levels, but the strength of selection at these levels is largely determined by physical, ecological, and social context. For example, colony-forming lineages are generally more likely to possess higher-level adaptations, whereas cell-level adaptations are more likely in populations of free-swimming cells. Current hypotheses for the adaptive function of toxins in microalgae often assume some degree of higher-level selection and should be evaluated against relevant aspects of the focal species' behavioral and ecology. This evolutionary perspective suggests novel, testable predictions associated with each class of hypothesis.

PALATABILITY DIFFERENCES BETWEEN TWO MORPHOLOGICALLY SIMILAR BLOOM-FORMING *ULVA* SPECIES

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In Narragansett Bay, Rhode Island, annual macroalgal blooms are dominated by two morphologically similar species: *Ulva pseudocurvata* and *U. rigida.* Field surveys conducted from 2009–2011 indicate that these species vary in abundance amongst months and sites, but neither species is consistently dominant within the blooms. To examine potential palatability differences between these species, we used image analysis to assess blade surface area throughout the summer of 2009. We found that *U. rigida* thalli had significantly more holes, suggesting that *U. rigida* is more palatable than its congener. Further evidence for this feeding preference was determined from in situ herbivore exclusion experiments conducted during 2010. Via principal component analysis, we found that *U. rigida* was consumed in greater quantities when mud crabs were the most abundant herbivore present. However, the underlying mechanism(s) for this preference remains unknown, as we have determined that *U. pseudocurvata* and *U. rigida* do not differ in thallus strength or nutrient levels, and *U. rigida* has higher levels of DMSP, which is typically associated with herbivore avoidance.

EFFECTS OF MACROALGAL CHEMICAL EXTRACTS ON SPORE BEHAVIOR OF THE ANTARCTIC EPIPHYTE *ELACHISTA ANTARCTICA*

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Most macroalgal species along the Western Antarctic Peninsula are defended against predation, many using chemical defenses. Also, the subtidal communities are mostly devoid of free-living filamentous algae. However, one endo/epiphyte, *Elachista antarctica*, is found growing exclusively out of the palatable rhodophyte *Palmaria decipiens.* In order to understand this unusual and exclusive epiphytization, we tested whether macroalgal secondary metabolites such as those responsible for deterring grazers also inhibit biofouling by the epiphyte. Swimming, settlement, and germination behaviors of the epiphyte's motile reproductive propagules were quantified in the presence of hydrophilic and lipophilic extracts of host *P. decipiens* and other sympatric rhodophytes. We hypothesize that the reproductive propagules responsible for *E. antarctica* dispersal cannot tolerate secondary metabolites of other possible hosts, chemotactically repelling spores away from the extracts as well as impairing their settlement and/or germination. Extracts from the normal host *P. decipiens* attracted *E. antarctica* spores. Chemoresponses to the different hydrophilic and lipophilic chemical extracts of other sympatric rhodophytes varied across the different algae tested, many of which did inhibit settlement, growth, or chemotactically repelled *E. antarctica* propagules.

HYDROCOLEUM IS PRIMARILY RESPONSIBLE FOR NITROGEN FIXATION IN TEXAS GULF COAST MICROBIAL MATS

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Microbial mats from sandy intertidal beaches of the Texas Gulf coast demonstrated substantial levels of nitrogenase activity. *Hydrocoleum* and *Microcoleus* dominated the upper green layer of mature mats.

Proto-mats (subsurface layers of cyanobacteria distinct from mature mats) contained almost exclusively *Microcoleus* and demonstrated no nitrogenase activity. *Hydrocoleum* was isolated as unialgal cultures. The level and diel pattern of nitrogenase activity and nitrogenase reductase (Fe-protein) were similar in intact mats and in isolated *Hydrocoleum* cultures. Primers and a probe specific for *Hydrocoleum nifH* along with q-rtPCR measurements, demonstrated similar patterns of expression in intact mats and cultures of isolated *Hydrocoleum*. Transcription and/or translation of *nifH* in *Hydrocoleum* may be controlled by circadian regulation since increased levels of *nifH* transcripts and Fe-protein appeared before the end of the light period of a diel cycle. In contrast, nitrogenase activity was only observed after the start of a dark period. Isolated cultures of *Hydrocoleum* formed mat-like structures. Their comparison with proto-mats and mature mats suggest that *Microcoleus* first colonizes sandy beaches, and then *Hydrocoleum* stabilizes mat structure, facilitating nitrogen fixation and mat structure.

PUTATIVE CASPASE ENZYMES, ACTIVITY, AND DOWNSTREAM DEATH SUBSTRATES ASSOCIATED WITH CHRONOLOGICAL AGING IN THE DINOFLAGELLATE *KARENIA BREVIS*

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Several putative caspase enzymes, key regulators in potentiating death signals through the programmed cell death (PCD) pathway, have been identified in *Karenia brevi*s through EST library searches and their active sites characterized to predict their substrate specificity. Quantification of caspase 3, 6, 7, 8, and 9 activities, determined by the specific cleavage of fluorogenic canonical substrates, demonstrated a significant increase during chronological aging, suggesting that the induction of caspase activity prior to culture decline may represent the utilization of the PCD pathway in executing death. Concurrently, global gene expression analysis demonstrated an extensive remodeling of the transcriptome which was indicative of a shift in the metabolic and signaling requirements during stationary phase. However, the expression level of transcripts for putative caspase enzymes did not change, agreeing with metazoan caspases which are regulated by post-translational activation of the enzyme. Computational prediction of downstream death substrates for the assayed caspase activities identified proteins involved in a wide range of biological processes including regulation of protein turnover, cell cycle progression, lipid metabolism, and autophagic cell death, lending new insight into the diversity of processes involved in the execution of death in dinoflagellates.

CHARACTERIZATION OF THE SPLICED LEADER RNA AND ITS RESPONSE TO STRESS IN THE SYMBIOTIC DINOFLAGELLATE, *SYMBIODINIUM MICROADRIATICUM*

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Spliced leader (SL) mediated trans-splicing has recently been identified in dinoflagellates, including the coral symbiont *Symbiodinium microadriaticum*. During SL trans-splicing, first described in trypanosomes, all mRNAs acquire an identical sequence at their 5′ end, donated from a snRNA, the SL. Under conditions of severe stress, trypanosomes shut off transcription of the SL gene, a response termed spliced leader silencing (SLS). SLS leads to a reduction of the available SL for trans-splicing and therefore, decreased mRNA maturation. This study sought to characterize the SL RNA in *Symbiodinium* and determine whether SLS is part of its stress response. The full-length SL transcript was amplified from total RNA, cloned, and sequenced. The SL RNA is 58-nt long with 67% identity to published dinoflagellate SL sequences. Using this sequence, a qPCR assay was developed to measure SL RNA expression. In contrast to trypanosomes, we found that SL RNA levels were not significantly different between control cultures and those exposed to either sublethal or lethal heat shock or 4 mM DTT. This is the first investigation into the functional role of the SL in dinoflagellates.

PHYTOCHEMICAL ANALYSES OF TOXIC POLYKETIDE METABOLITES FROM *PRYMNESIUM PARVUM* CARTER (HAPTOPHYTA)

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Hemolytic effects of polyketide metabolites from *Prymnesium parvum* were examined as a function of salinity and culture age. Polyketide prymnesins were verified by metabolic fingerprint analysis (HPLC/MS). Total hemolytic activity (HA) of culture supernatants and cell extracts was measured. Variation in supernatant HA was statistically significant relative to both variables. Salinity was inversely related to HA;

whereas HA of supernatants was inversely related to culture age during the first 3 weeks, but positively related to it during the next 3 weeks. Interestingly, no hemolysis was detected in prymnesin-enriched fractions from supernatants, as the majority of hemolysins remained in other fractions. The HA of cell extracts had the exact opposite correlation pattern. Salinity was directly related to HA from cell extracts, particularly during the latter 3 weeks. These investigations suggest that extracellular polyketide prymnesins are present at much lower concentrations than previously believed and they are not the major compounds associated with hemolysis under these conditions.

THE ROLE OF REACTIVE OXYGEN SPECIES IN ANTI-HERBIVORE DEFENSE ALONG THE WESTERN ANTARCTIC PENINSULA

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Cellular production of reactive oxygen species (ROS) is involved in the innate immunity of algae, but the idea that they may also be involved in direct defense against macroscopic predators is new. Macroalgae along the Western Antarctic Peninsula grow in the presence of large communities of mesograzers, particularly amphipods. Macroalgal species employ a range of defenses including secondary metabolite chemistry, physical toughness, and low nutritional content. However, these factors cannot always account for mesograzer deterrence. Interestingly, ROS have been implicated in the wounding response for several temperate algal species. We hypothesize that ROS produced during the Antarctic macroalgal wound response serve as a component of an activated chemical defense against grazing. We found that ROS production upon wounding varied widely among the 13 macroalgal species studied. ROS release was substantially higher in *Palmaria decipiens* than other species, and P. decipiens is the only species studied that is truly palatable to the sympatric amphipod *Gondogeneia antarctica*, as it lacks secondary metabolites and toughness and is nutritionally rich. *G. antarctica* responded to micromolar concentrations of exogenous hydrogen peroxide with a reduction in feeding rate. Our findings suggest that *P. decipiens*, undefended by secondary metabolic, physical, or nutritional factors, may use ROS to decrease amphipod grazing.

RED-TIPPED *LEPTOLYNGBYA* (CYANOBACTERIA) SPECIES OF DESERT HABITATS IN THE AMERICAS

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A group of *Leptolyngbya* species containing reddish granules (rhodopsin) in the tips of apical cells of trichomes has been found in caves in Europe, and has been under study for over a decade. Recently, we isolated red-tipped *Leptolyngbya* strains from desert habitats that belong to the same clade as the cave-dwelling forms. Three populations are represented from: soil of the Atacama Desert, Chile, soil of the Mojave Desert of California, and a seep wall in Utah. The strains of these three localities have been isolated into culture and characterized in the light microscope. Growth experiments with varying light levels indicate growth in all three strains is better at lower intensity, and that the rhodopsin is photodegraded at high light. The two populations from desert soil are morphologically and genetically similar and distinct from the Utah strain (which has a smaller diameter and different 16S–23S ITS). Phylogenetic analysis based on 16S rRNA gene sequences indicates that the red-tipped *Leptolyngbya* form a clade outside of *Leptolyngbya sensu stricto*, and therefore need to be recognized as new species in a new genus.

ESTABLISHING SPECIES LIMITS AND PHYLOGENETIC RELATIONSHIPS FOR NORTHWEST ATLANTIC *POLYSIPHONIA* (RHODOPHYTA) USING AN INTEGRATED TAXONOMIC APPROACH

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DNA barcoding, using COI-5P, has an established track record for the rapid and accurate assignment of red algal specimens to species groups. Used in combination with careful morphological observations and additional molecular markers (e.g., *rbc*L), accurate species delimitation and designation can be completed for taxonomically challenging genera. *Polysiphonia* (Rhodomelaceae) is a large and widely distributed genus of red algae, which, despite considerable taxonomic study, has a number of uncertainties with regards to species richness and respective biogeography in North America. In this study species diversity for the genus *Polysiphonia sensu lato* (including *Neosiphonia* and *Vertebrata*) in the northwest Atlantic was investigated. Currently, there are twelve species of *Polysiphonia* reported from this region, however, we identified fourteen unique genetic species groups through COI-5P and *rbc*L sequence data. Based on current floristic guides multiple genetic groups key out to the same morphological species, and, despite extensive sampling, we have not collected four of the twelve currently recognized morpho-species. These results indicate that there is hidden species diversity within the genus *Polysiphonia* in the northwest Atlantic and that substantial taxonomic work remains.

REMARKABLE GENETIC AND SILICA-SCALE DIVERSITY IN THE COLOURLESS CHRYSOMONAD, *PARAPHYSOMONAS*: TAXONOMIC AND EVOLUTIONARY IMPLICATIONS

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Silica scale morphology of protists has been an anchor-point in the description of new species since the electron microscope first allowed. Many descriptions of new species of *Paraphysomonas*, a colourless ochrophyte (Chrysophyceae), come from electron microscope environmental surveys of un-cloned cells. I have isolated and sequenced 52 new clonal isolates of *Paraphysomonas*; observed their silica-scales, cell dimensions, and 18S rDNA phylogeny, to uncover diversity beyond scale morphology. I have observed that there is a common and widespread occurrence of spine scales within this genus that separates into distinct groups on the 18S trees. The genetic diversity of all the spine scale isolates is notable. Numerous lineages share broadly similar scale morphologies and are genetically distinct. Subtle differences in spine-scale morphology map well onto phylogenetic trees for which evolutionary and taxonomic theory can be made.

My data strongly indicate a far greater diversity within this group, arguably doubling the amount of known species. The commonness of some species is probably the result of excessive lumping of genetically distinct species that have similar scales thus indicating a need for further characterisation.

A NEW *BRASILONEMA* (CYANOBACTERIA) SPECIES FROM THE HAWAIIAN ISLANDS WITH AN EMENDATION OF THE GENUS

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A new species of the genus *Brasilonema* was isolated from the island of Oahu, Hawaii. The taxon is distinguished from the seven other species in the genus by attenuation of trichomes, and the intended name is ‘‘*Brasilonema angustata*’’. It possesses the cytoplasmic kerotimization and reddish-brown coloration of several species in the genus. Phylogenetic analysis based on 16S rRNA gene sequence shows ‘‘*B. angustata*’’ within a highly supported clade containing all sequenced *Brasilonema* species. We compared the secondary structure of the 16S-23S ITS regions for *B. octagenarum* and ‘‘*B. angustata*’’. The structurally conservative D1-D1’ and V3 helices show similar motifs between the two taxa, but differ structurally and in sequence, providing additional justification for erection of the new species. The Box-B helix has identical secondary structure. The existence of tapering in a *Brasilonema* species requires emendation of the genus description of *Brasilonema*, which was described as unattenuated. Our phylogenetic evidence supports the hypothesis that tapering has developed repeatedly in separate cyanobacterial lineages and lacks the taxonomic significance once assumed by early workers.

POLYPHYLY OF *CHAETOPHORA* AND *STIGEOCLONIUM* AND NEW INSIGHT INTO THE CBC-CLADE SPECIES CONCEPT WITHIN THE CHAETOPHORALES (CHLOROPHYCEAE, VIRIDIPLANTAE)

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To enhance limited knowledge about phylogenetic relationships among families/genera/species within the Chaetophorales (Chlorophyceae, Viridiplantae), newly obtained SSU and ITS2 rDNA sequences have been investigated in detail. Phylogenetic analyses revealed (1) a basal phylogenetic position for the Schizomeridaceae and the weakly-supported Aphanochaetaceae, and (2) polyphyly of the species-rich genera *Chaetophora* and *Stigeoclonium*. The demonstrated polyphyly of both genera has been further supported by molecular unique synapomorphies (NHS) in the SSU rRNA secondary structure within the Viridiplantae. To gain insights into ITS2 evolution and the CBC-clade species concept, all substitutions have been mapped upon the ITS2 phylogeny. Furthermore, high sequence and length conservation allowed the establishment of an ITS2 consensus secondary structure, and application of a novel numbering system of ITS2 nucleotides/base pairs. The comparative analyses of ITS2 (1) revealed surprisingly low number of Compensatory Base Changes (CBCs) in Helix 2 and/or 3 within the Chaetophorales and (2) demonstrated several discrepancies between ITS2 evolution in the Chaetophorales and the generally accepted CBC-clade concept *sensu* Coleman.

SPECIES DIVERSITY AND MOLECULAR PHYLOGENY OF FOLIOSE *GRACILARIA* (GRACILARIACEAE, RHODOPHYTA) IN THE WESTERN PACIFIC OCEAN

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Foliose species of *Gracilaria* are common red algae found in the western Pacific Ocean. Previous studies have shown that foliose species with textorii-type spermatangial conceptacles have two types of thallus morphology: blades with spiny margins (e.g. *G. vieillardii, G. spinulosa, G. huangii*) and blades with smooth margins (e.g. *G. textorii*, *G. incurvata*). Our preliminary molecular analyses show that species diversity among foliose *Gracilaria* is much higher than previously thought and there are several undescribed, cryptic species, which were previously regarded as either *G. vieillardii* or *G. textorii*, present in the warm water regions. In this study, we will describe the diversity of the foliose species found in the western Pacific Ocean and infer their inter-specific relationships based on rbcL sequence analysis. The significance of morphological characters for separating foliose species, such as thallus morphology, blade thickness, number and distribution of tubular nutritive cells inside the cystocarp cavity, and the formation of the spermatangial conceptacles and the carposporangia, will be discussed.

DESCRIPTION OF A NEW SPECIES OF *VOLVOX* SECT. *VOLVOX* FROM JAPAN BASED ON COMPARATIVE MORPHOLOGY AND MOLECULAR PHYLOGENY

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Volvox sect. *Volvox* (Volvocaceae, Chlorophyceae) has unique thick cytoplasmic bridges between cells in spheroids and spiny-walled zygotes. This section is taxonomically important because the genus *Volvox* is polyphyletic. However, taxonomic studies of species in *Volvox* sect. *Volvox* have not been carried out since Smith (1944). In Japan, only *V. globator* was recorded in this section based on a very classic study by Ishikawa (1896, Prof. Zoo. Coll. Agri. Imp. Univ.). In order to elucidate the correct species diversity of this section, we recently commenced the comparative morphology and molecular phylogeny using cultured strains. Here we show a new species of this section, *V. ferrisii* Isaka et al. sp. nov. This species can be distinguished from other monoecious species of *Volvox* sect. *Volvox* based on the differences in numbers of zygotes (eggs) in the sexual spheroids, form of zygote wall and somatic cell shape. Sequences for ITS of nuclear *r*DNA resolved that the new species has phylogenetic positions separated from *V. globator, V. barberi, V. capensis* and *V. kirkiorum* within *Volvox* sect. *Volvox.*

MOLECULAR IDENTIFICATION OF RICKETTSIACEAN ENDOSYMBIONTS IN THE VOLVOCALEANS, *CARTERIA CERASIFORMIS* AND *PLEODORINA JAPONICA* (CHLOROPHYTA)

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Endosymbiotic bacteria were observed by TEM in the cytoplasm from several strains of the freshwater

volvocalean greens (e.g. Kochert & Olson (1970); Nozaki et al. (1989). However, phylogenetic positions of these bacteria have remained unresolved. Here, we performed a molecular phylogenetic analysis of bacterial endosymbionts harbored in cells of unicellular *Carteria cerasiformis* NIES-425 and colonial *Pleodorina japonica* NIES-577. Phylogenetic analysis based on 16S *r*RNA sequences demonstrated that endosymbionts from these two volvocaleans and marine ciliate *Diophrys appendiculata* formed a small group that was placed within the family Rickettsiaceae, an obligate intracellular parasitic bacterial group. Such endosymbionts were not detected in 10 other strains of the closely related four *Carteria* species, based on DAPI-staining and genomic PCR using Rickettsiaceae-specific primers newly designed. These results suggest a possible horizontal transmission of the endosymbiont to *C. cerasiformis* NIES-425. This is the first report of endosymbiotic bacteria from the family Rickettsiaceae inside eukaryotic algal cells. In situ hybridization demonstrated existence of bacteria from the family Rickettsiaceae in the volvocalean cells. Therefore, we will propose provisional names for these endosymbionts.

SPIROGYRA AND *SIROGONIUM* (ZYGNEMATACEAE, CHAROPHYTA) IN HAWAI'I: MOLECULAR INSIGHTS INTO TWO MORPHOLOGICALLY SIMILAR FRESHWATER MACROALGAL GENERA

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Spirogyra is a filamentous algal genus with >380 species found in freshwater systems worldwide. Despite its prevalence, there is much taxonomic confusion between *Spirogyra* and the closely related and morphologically similar genus, *Sirogonium.* DNA sequences of selected regions of cp23S rRNA (UPA), *rbcL,* and nu18S were obtained from 151 isolates of *Spirogyra* and putative relatives in the Hawaiian Islands. Individual and concatenated sequence data sets were analyzed using maximum parsimony, maximum likelihood and Bayesian inference. Morphological characters were also measured and analyzed for each collection, and compared to results based on sequence data. Twelve species of *Spirogyra/Sirogonium* were identified in the *rbcL* trees, and as in previously published analyses, *Spirogyra* and *Sirogonium* are paraphyletic. This first molecular phylogenetic study of relationships within *Spirogyra* and *Sirogonium* in the Hawaiian Islands contributes important data on variation among the most isolated of all *Spirogyra/Sirogonium* populations in the world and thus the groundwork for a better understanding of these genera worldwide.

POST-TRANSCRIPTIONAL REGULATION OF THE CELL CYCLE IN THE RED TIDE DINOFLAGELLATE, *KARENIA BREVIS*, AND A POTENTIAL ROLE FOR CYCLIN-DEPENDENT KINASE

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Karenia brevis produces harmful algal blooms that cause marine animal mortalities and human illness. Molecular mechanisms controlling cell cycle entry in *K. brevis* are important because bloom development occurs through vegetative cell division. Studies have demonstrated that, unlike typical eukaryotes, dinoflagellate cell cycle genes are not regulated at the transcriptional level. Post-transcriptional control of these genes is also suggested by the presence of a trans-spliced leader sequence on their transcripts. The abundance of replication fork proteins over the cell cycle was investigated to determine whether they are regulated at the translational or post-translational level. The replication fork proteins, PCNA, RFC, RPA and RnR2 change over the cell cycle with highest abundance at S-phase, suggesting translational control. PCNA also appears to be modified post-translationally, either by ubiquitin or SUMO concurrent with S-phase. PCNA is present in the nucleus throughout the cell cycle in cells actively traversing the cell cycle. PCNA showed a pattern of nuclear location that changes between a chromatin bound form and a pool that is peripheral. Cell cycle inhibition at G1/S with the CDK4 specific inhibitor, fascaplysin or the pan-CDK inhibitor olomoucine specifically inhibited the expression of PCNA protein. Here, we propose a novel mechanism of translational control of cell cycle entry as opposed to transcriptional control which is seen in most eukaryotes.

A MOLECULAR PHYLOGENETIC STUDY OF THE TRIBE CORALLINEAE (CORALLINALES, RHODOPHYTA) WITH AN ASSESSMENT OF GENUS-LEVEL TAXONOMIC FEATURES

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A multi-gene phylogeny using CO1-5P (cytochrome oxidase subunit 1), psbA (photosystem 1) and EF2 (nuclear elongation factor) sequence data for members of the tribe Corallineae (Corallinaceae, Corallinales, Rhodophyta) was constructed to assess generic boundaries. We determined that traditional reliance on conceptacle position as an indicator of generic affinities in the Corallineae is not supported and taxonomic changes are required. Notably, the genus *Serraticardia* was polyphyletic; we propose synonymy of *Serraticardia* with *Corallina*, effecting transfer of the type species *S. maxima* to *Corallina* (*C. maxima* (Yendo) comb. nov.). We propose the new genus *Johansenia* for *S. macmillanii* (*J. macmillanii* (Yendo) comb. nov.), which does not have affinities with *Corallina*. Our molecular data also indicate that the genus *Marginisporum* has evolutionary affinities among species of *Corallina* and these genera should be synonymized, *Corallina* having nomenclatural priority. Thus, we propose the new combinations *Corallina aberrans* (Yendo) comb. nov., *Corallina crassissima* (Yendo) comb. nov., and *Corallina declinata* (Yendo) comb. nov. *Corallina elongata* was divergent from all other members of the *Corallina* clade and is also transferred to a new genus, *Ellisolandia*.

THE IMPACT OF ENDOPHYTE PRESENCE ON GROWTH AND SURVIVAL IN ANTARCTIC MACROALGAL HOSTS (RHODOPHYTA)

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Filamentous algae along the Western Antarctic Peninsula are primarily restricted to an endophytic lifestyle within macroalgal hosts, apparently because of heavy mesograzer pressure on free-living filamentous algae. The presence of endophytes may have compounding effects on host macrophytes due to competition for light and nutrients, or potential pathogenic effects. This study focused on growth and survival of individuals with varying levels of endophyte infection within populations of three macrophytic red algae around Palmer Station, Antarctica. Individuals were collected from the field, weighed and photographed in the lab, and out-planted back to their natural environment for six or nine weeks at the end of the Austral summer, 2010. Growth was measured in four ways: weight change, change in surface area, growth rate, and surface area corrected growth rate. Survival was measured by loss of whole individuals. Increased endophyte load significantly affected growth rate, weight change, and survival in *Gymnogongrus turquetii*, as well as growth rate in *Trematocarpus antarcticus*. The presence of reproductive structures was observed to increase with endophyte presence in *Myriogramme mangini*.

MOTILITY DURING PELAGIC-BENTHIC LIFE-STAGE TRANSITIONS FOR THE HARMFUL ALGA, *HETEROSIGMA AKASHIWO* AND THE IMPLICATIONS FOR BLOOM DYNAMICS

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In many harmful algal blooming (HAB) species, transitions between a motile, pelagic vegetative stage and a non-motile, benthic resting stage strongly impact HABs. Swimming during life-stage transitions may be used to actively influence vertical fluxes, and hence, population-level distributions. However, these transitional swimming behaviors are poorly understood. We used video-based motion analysis to quantify swimming of the HAB-forming alga *Heterosigma akashiwo* during pelagic-benthic transitions. Our video assay accurately assigned cells to one of three physiological states observed during transition to the resting stage based on statistical characteristics of individual paths. Net vertical velocities of individuals in each state showed no evidence of down-swimming, indicating cells reach the sediments by sinking. Outputs from our numerical water column model suggested active down-swimming might be disadvantageous to deposition in benthic habitats suitable for re-emergence relative to sinking. As many as 26% of laboratory-formed resting cells regained the up-swimming ability necessary for bloom formation within 24 h of transitioning from resting cell induction (10 C; dark) to growth-supporting(15 C;12 h light:12 h dark) conditions. Our video-based methods are being implemented as remote sensing for the prediction of HABs.

CONTRIBUTED TALKS

A TYPICALLY UNUSUAL DINOFLAGELLATE MITOCHONDRIAL GENOME AND AN UNUSUALLY TYPICAL DIATOM MITOCHONDRIAL GENOME CONSTITUTE THE MITOCHONDRIAL GENOMES OF ''DINOTOMS''

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We have characterized the nearly complete coding sequences of the complex and evolutionarily dissimilar mitochondrial genomes in *Durinskia baltica* and *Kryptoperidinium foliaceum*, two dinoflagellates with a diatom endosymbiont. The endosymbiont mitochondrial coding sequences of *D. baltica* (34242 bp) and *K. foliaceum* (34742 bp) have identical gene content (shared with other diatoms) and gene order. Events such as gene-splitting, gene-fusion, and in-frame insertion have occurred in the endosymbiont's mitochondrial genomes, also seen in the diatom *Phaeodactylum tricornutum.* From *D. baltica,* we have sequenced several fragments of dinoflagellate *lsurRNA*, many fragmented forms and one complete copy of the only three protein-encoding genes interspersed within stretches of non-coding and repeated elements. The complete *cox1* copy is uninterrupted, whereas *cob* and *cox3* appear in two separate pieces necessitating trans-splicing. From *K. foliaceum* we have only found parts of all the three protein-encoding transcripts. Overall, our results indicate that these two distinct mitochondrial genomes in each ''dinotom'' have continued to follow their respective evolutionary paths (just like their counterparts in free-living diatoms and dinoflagellates) despite their long coexistence side by side within a single cell.

A SMALL GENOME THAT ''THINKS BIG'': THE COMPLETE NUCLEOMORPH GENOME OF THE CRYPTOPHYTE ALGA *CHROOMONAS*

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The cryptophytes are a diverse lineage of secondary plastid-containing algae. In most secondary plastid-containing lineages, reduction of the secondary endosymbiont has gone to completion. In the cryptophytes, however, the endosymbiont nucleus, the nucleomorph, persists. Nucleomorphs are the smallest eukaryotic nuclear genomes known, having undergone extensive genomic streamlining through mechanisms such as gene loss and endosymbiotic gene transfer. Whether or not nucleomorphs have reached an endpoint or are still undergoing reductive evolution is unknown. To address this question, we completely sequenced the 685 Kbp nucleomorph genome of the cryptophyte *Chroomonas mesostigmatica* CCMP1168, the largest nucleomorph genome sequenced to date. In stark contrast to other cryptophyte nucleomorph genomes, the nucleomorph genome of *C. mesostigmatica* contains more (and larger) genes and spliceosomal introns, larger intergenic regions, multi-copy genes, and repetitive elements, all features that contribute to its larger genome size. The *C. mesostigmatica* nucleomorph genome, while similar in gene content to other cryptophyte nucleomorph genomes, is by far the most ''complex'' nucleomorph genome studied to date, exhibiting features more characteristic of its presumed free-living red algal relatives.

POSITIVE SELECTION IS DETECTED WITHIN A DIATOM SPECIES AND AFFECTS REGULATORY GENES

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Natural selection acts on individuals affecting adaption to changing environmental conditions, and is measured as positive selection at the genetic level using the ratio of amino acid (dN) changing mutations to silent mutations (dS). Genome and transcriptome-wide pair-wise comparisons of three diatom genera were made, testing the phylogenetic distance at which positive selection (dN:dS>1.0) can be detected. Detection of positive selection was weakest among three species of *Pseudo-nitzschia,* but increased between cryptic sister-species of *Ditylum brightwellii.* The greatest number of genes likely to be positively selected was detected for two strains of *Thalassiosira pseudonana.* Further testing among seven globally distinct strains of *T. pseudonana* yielded between 809 (Bonferroni corrected) and 1,784 (false discovery rate corrected) positively selected genes, representing 7 to 16%, respectively, of the known protein coding genes. Functional enrichment, associated with stress

tolerance and regulation of the cell cycle and transcription, was found for 146 of the 809 genes. Differential selection was not found among individual lineages, suggesting that the selected genes act broadly on the species, facilitating adaptation to unpredictable estuarine and oceanic conditions.

DIVERSE ORIGINS OF HETEROKONT GENES – NEW INSIGHTS FROM A DICTYOCOPHYTE EST LIBRARY

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Diverse origins of genes and gene families in heterokonts have been much debated, and several horizontal gene transfers/endosymbiosis events have been proposed to explain them. Here we present the analysis of an EST library from the unicellular heterokont *Pseudochattonella farcimen* (Dictyochophyceae), revealing the presence of several haptophyte-like fatty acid desaturases in this organism. Together with recent analyses of photosynthesis-related genes, this finding supports the hypothesis of an origin of *Pseudochattonella* plastids from an endosymbiosis event involving a haptophyte, or another horizontal gene transfer between these lineages. Our analysis also shows *P. farcimen* to contain genes of the mannitol cycle, which were proposed to have been acquired by brown algae from bacteria. Screening of published genomes and EST libraries, and measurements of mannitol concentrations in a range of different heterokonts demonstrated that this transfer probably took place earlier than expected, in a common ancestor of brown algae and dichtyochophytes. Additionally, genes such as type I polyketide synthases may also have been acquired simultaneously, but later lost in some classes. A minimal model required to plausibly explain these observations is presented.

ACTIVE RELEASE OF SEQUESTERED CARBON BY DIURNALLY SYNCHRONIZED PROGRAMMED CELL DEATH OF MICROALGAE

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Algal photosynthesis accounts for 50% of the total organic carbon (C) produced annually. We have discovered that photosynthetically sequestered C is released by the eukaryotic unicellular chlorophyte *Dunaliella salina* through synchronized programmed cell death (PCD) of 40–74% of its own population. In turn, we observed that the algal population recovers at a remarkable rate, increasing in size with up to three doublings upon onset of daytime. We explored the ecological relevance of these phenomena by tracking the flow of C in co-cultures of *D. salina* and *Haloarchaea salinarum,* a co-inhabitant in the natural environment that, is able to independently induce PCD, decelerating algal population growth. This has revealed an interesting mutualism in which the algal photosynthate complements nutritional needs of haloarchaea, who remineralize the C and effect a net increase in algal productivity. Induction of specific haloarchaeal transcriptional programs by the algal photosynthate suggests that several mechanisms have co-evolved to support the interplay between the two organisms. This discovery brings to fore an important role for active programmed release of cell constituents in driving nutrient cycles through the microbial loop.

PROFILING OF THE TRANSCRIPTOME OF *PORPHYRA YEZOENSIS* WITH SOLEXA SEQUENCING TECHNOLOGY

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With high-throughput Solexa sequencing technology, we profiled *Porphyra yezoensis* transcriptomes from eight different samples. More than 1,200 megabases from 13,333,334 quality paired-end reads were generated, which were assembled into 31,538 unigenes. Blast analysis showed that 56.7% unigenes were novel, which represented the specific genes of *Porphyra* and/or rhodophytes. Several hundreds of unigenes related to stress tolerance were discovered, including desiccation- (211) and high light-tolerance (31), MAAs and flavonoid biosynthesis (10), reactive oxygen scavenging (48) and others (208), which indicated there existed complex and diversity modes of stress-tolerance in this species. A complete set of essential genes involved in C3- (57) and C4- (44) carbon fixation pathway (except pyruvate phosphate dikinase) were discovered, which not only proved that they were actively transcribed but clearly outlined the panoptic view of carbon fixation in *Porphyra*. Moreover, by statistically analyzing the types, proportions and frequencies of the interspersed repeats (TEs) and simple sequence repeats (SSRs), we discovered that the top three types of TEs were all the retrotransposons and the trinucleotide is the absolute predominant type among SSRs, promoting our understanding of structural characteristics of the transcriptome. This study substantially improved the global view of the *Porphyra* genome and provided a valuable resource for future research.

POPULATION GENOMICS OF MICROSPORIDIAN PARASITES

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Comparative genomics of microbial eukaryotes has revealed some of the breadth of genome diversity and trends that underpin it. Comparisons between complete genome sequences from closely related species remain rare, however, so equally interesting shorter-term processes remain less clear. We have taken advantage of the exceedingly small and compact nature of nuclear genomes of obligate intracellular microsporidian parasites to examine these processes at one extreme of genome diversity. Encephalitozoon genomes are typically only 2–3 Mbp. The *E. cuniculi* genome (2.9 Mbp) was sequenced a decade ago, and we recently sequenced the genomes of *E. intestinalis* (2.3 Mbp), two strains of *E. hellem*, and three strains of *E. cuniculi* to allow finer-scale comparisons. Inter-species comparisons reveal unexpected differences, including the acquisition of blocks of functionally-related genes by horizontal gene transfer, some likely from host animal genomes. Intra-species comparisons revel short-term changes in progress, including gene duplication and pseudogenization at high frequencies in specific regions of the genome, as well as patterns of single nucleotide polymorphisms that can indicate whether events such as sexual recombined have taken place between strains.

DEVELOPMENT OF MOLECULAR INDICATORS FOR THE IRON NUTRITIONAL STATUS OF DIATOMS

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We have developed molecular-based approaches to assess the iron nutritional status of diatoms. Our initial efforts focused on *Pseudo-nitzschia*, a pennate diatom genus of which members often exhibit large growth responses to iron fertilization in iron-limited regions. The expression patterns of two genes track the iron status of *Pseudo-nitzschia granii* with high fidelity. The first gene, FTN, encodes for the specialized iron-storage protein ferritin and is highly expressed under iron-replete conditions. The second gene, ISIP2A, encodes for an iron-starved induced protein of unknown function and is highly expressed under iron-limiting conditions. The resulting ISIP2A:FTN transcript ratio is reflective of the iron status of the cells. Growth of *P. granii* under different environmental conditions has verified that the transcript ratio is primarily influenced by iron status. Using a combination of next-generation sequencing technologies, we also performed a comparative transcriptome of *P. granii* under varying iron conditions, obtaining over 2,400 differentially expressed genes. In conjunction with the select gene approach we propose the use of comparative metatranscriptomics as an effective means to deciphering the nutritional status of phytoplankton within natural assemblages.

EXTENSIVE GENE TRANSFER IN *BLASTOCYSTIS* SP., NANDII STRAIN

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Lateral gene transfer (LGT), the transfer of genetic information between distantly related organisms, is now known to be a significant force in prokaryotic genome evolution. The recent explosive growth of genomic data from diverse eukaryotic lineages revealed that LGT also occurs in eukaryotes. *Blastocystis* spp. NandII strain is an anaerobic parasitic stramenopile that infects humans preferentially. To study the genetic composition of this strain of *Blastocystis*, we performed transcriptomic surveys. Here we report our analyses of this dataset aimed at evaluating the impact of LGT on the *Blastocystis* spp. NandII proteome. Focusing on *Blastocystis* sp. homologs, we used a series of automated methods for alignment generation, followed by maximum likelihood phylogenetic estimation. Out of 2,340 phylogenetic trees, approximately 20% showed evidence of ''lateral'' origin in *Blastocystis* and included genes of archaeal, proteobacterial, algal and fungal affinities. The genes of apparent LGT origin were mainly of informational nature. Amongst these, we identified genes that play roles in key biosynthetic pathways, including the iron-sulfur cluster pathway, sugar and purine biosynthesis as well as protein trafficking and ubiquination.

THE HARMFUL ALGA *AUREOCOCCUS ANOPHAGEFFERENS* USES TWO SEPARATE XANTHOPHYLL STRATEGIES FOR PHOTOPROTECTION DURING HIGH LIGHT ACCLIMATION

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Aureococcus anophagefferens is a picoplanktonic microalga that causes devastating blooms in estuarine waters. It can reach maximum growth rates at lower light intensities than competing species of diatoms and is endowed with at least 60 genes encoding fucoxanthin Chl a/c proteins (FCPs). However, four of these genes belong to the Lhcx sub-clade, known to be involved in photoprotection rather than light-harvesting in other algae. We analyzed *Aureococcus* under low (LL,20 mmol photons m-2s-1), medium (ML, 60 or 90 mmol photons m-2s-1) and high (HL, 200 mmol photons m-2s-1) light and during a 1 or 6 h shift to higher light intensities. The increase in both diadinoxanthin and diatoxanthin was correlated with the appearance of three new FCPs (21, 24 and 34 kDa), suggesting they might be binding these carotenoids. There was also a marked increase in the ratio of 19'-butanoyloxy-fucoxanthin (19BFx) to fucoxanthin (Fx). We suggest 19BFx could make energy transfer less efficient, thus contributing to photoprotection. It also suggests caution in using 19BFx as a quantitative marker for pelagophytes in ocean sampling.

METAGENOMICS OF *EUGLENA EHRENBERGII*

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Using Solexa/Illumina sequencing method and assembling by the Velvet program we have obtained the substantial portion of the chloroplast genome of *Euglena ehrenbergii* comprising entire sequences of LSU and SSU rDNA, 25 tRNA genes and 49 protein coding genes. Additionally, we have assembled almost entire genome of the bacteria *Pirellula staleyi* and obtained contigs of several other bacterial genomes, some known and some not, apparently present in *E. ehrenbergii* culture. We also assembled about 95% of cytoplasmic SSU and 85% of cytoplasmic LSU genes from *Euglena ehrenbergii.* The sequences of four rRNA genes (cytoplasmic and chloroplast) were used to establish the phylogenetic position of *E. ehrenbergii* within Euglenales and sequences of 16S rDNA were used to obtain the phylogeny of *E. ehrenbergii* associated bacteria.

A GEMINI VIRUS-LIKE REPLICATION PROTEIN WITHIN A XANTHOPHYTE CHLOROPLAST GENOME

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The chloroplast genomes of two Xanthophyte representatives have been sequenced. *Botrydium cystosum* (Botrydiales) is a large, globose organism often found on a soil substrate whereas the fine filaments of *Tribonema aequale* (Tribonematales) are aquatic. Unexpectedly, the chloroplast genomes of these two algae are remarkably similar, having a highly conserved architecture with limited rearrangements as well as a nearly identical gene composition. Several unique genes are encoded in the chloroplast genomes of these Xanthophytes. Most intriguing is the presence of a Gemini virus-like replication initiator protein that is encoded in the *B. cystosum* chloroplast genome. Gemini viruses replicate via a rolling circle mechanism and the replication initiator protein is indispensable to this process. Important amino acid motifs, characteristic of replication initiator proteins, have been identified in the *B. cytosum* protein. These functional domains are responsible for DNA-protein interaction, metal binding, and catalysis. Like replication proteins in bacterial plasmids, the *B. cystosum* protein lacks both Walker A and B domains, indicating the absence of helicase activity. A similar replication protein has been reported in a plasmid that infects *Porphyra pulcara* (Rhodophyceae).

EXPRESSION PROFILING OF LIGHT HARVESTING COMPLEXES IN ENVIRONMENTAL SAMPLES OF THE GIANT KELP, *MACROCYSTIS PYRIFERA*

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The Giant Kelp, *Macrocystis pyrifera*, possesses several antenna pigment complexes (LHC). Single celled algae can optimize light absorption under a variable light availability through differential expression of different light harvesting complexes. An individual *Macrocystis* can reach heights of 10s of m and can perform photosynthesis under a wide range of light levels. This may potentially be achieved through the differential expression of LHC. We used 454 pyrosequencing technology from four depth-stratified and seasonally-separated cDNA libraries to generate gene models for *Macrocystis*, including those for LHC, and to quantitatively survey expression profiles. BLAST searches of the transcriptome assembly identified nine putative LHC contigs (MpLHC). A phylogenetic analysis of the MpLHC and the LHC genes from other heterokonts grouped the MpLHC into canonical LHC groups I, II/III, and IV and L1818. The MpLHC displayed different expression patterns with depth and between seasons with the L1818 family showing the greatest transcriptional variation between the surface and depth, consistent with its hypothesized role in non-photochemical quenching. The expression patterns of the MpLHC were further examined using Q-PCR on finer resolution depth profiles.

CHARACTERIZATION OF THE *ALEXANDRIUM* TRANSCRIPTOME REVEALS UNIQUE NUTRIENT UTILIZATION STRATEGIES IN THE DINOFLAGELLATE

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The bloom-forming dinoflagellate *Alexandrium* is common in many near-shore marine ecosystems and causes paralytic shellfish poisoning through the production of saxitoxin. Genome sequencing of an *Alexandrium* species would significantly aid researchers in their search for genes involved in toxin production, bloom dynamics, and other critical metabolic pathways; however, giant genome sizes in the dinoflagellates (100 gb in *Alexandrium*) makes a genome sequence from this ecologically and economically important organism presently impractical. To avoid this bioinformatics hurdle, we sequenced 9.8 gb of transcriptomic cDNA from *Alexandrium tamarense* CCMP1598. The data assembled into 101,118 contigs, which rarefaction analysis suggests represent the vast majority of the species' gene repertoire and the largest collection of dinoflagellate sequences currently

available. We compared the *A. tamarense* transcriptome to the genomes of representative microbial marine eukaryotes and identified genes unique to the dinoflagellate including genes involved in nutrient utilization such as nitrogen, carbohydrate, and lipid metabolism. Phylogenomic analysis reveals that many of these genes appear to be recently acquired from bacteria suggesting horizontal gene transfer is a significant driver of gene evolution in this dinoflagellate.

EVOLUTION IS VARIED AMONG MITOCHONDRIAL GENES IN THE RAPHIDOPHYTE, *HETEROSIGMA*

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Unicellular algae are important primary producers in aquatic systems, yet can be ecologically devastating when blooms form. Many cells of the same species are indistinguishable morphologically; yet contain genetic variation that confers distinct physiological capacities that may impact bloom severity. The raphidophyte alga, *Heterosigma*, found worldwide, is an example of one such cryptic species complex. To analyze potential cryptic speciation in *Heterosigma*, 50 cultured strains were genetically fingerprinted. Sequences from five mitochondrial genes were used to probe genetic diversity. Combinations of resulting unique mitochondrial gene sequences resulted in the identification of 23 mitotypes. Some mitotypes appear ‘‘mixed’’, containing genes from two types – implying recombination through deep coalescence initiating from heteroplasmic organelles, or introgressive hybridization. This observation casts a cautionary light on interpretation of data when only one mitochondrial gene is used to infer evolutionary relatedness. In our study, we show that little geographic fidelity occurred among mitotypes, and multiple mitotypes coexist in the same local. The theoretical impact imposed on mitochondrial activity through amino acid changes was probed by modeling protein function scores for each mitotype.

DYNAMICS OF GENE DUPLICATION IN THE GENOMES OF CHLOROPHYLL D-PRODUCING CYANOBACTERIA: IMPLICATIONS FOR THE ECOLOGICAL NICHE

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Gene duplication may be an important mechanism for the evolution of new functions or the adaptive modulation of gene expression via dosage effects. We analyzed the fate of gene duplicates for two strains of a novel group of cyanobacteria (genus *Acaryochloris*) that produces chlorophyll d as its main photosynthetic pigment. The genomes of both strains contain an unusually high number of gene duplicates for bacteria. Most duplicated *Acaryochloris* genes are of comparatively recent origin, are strain-specific and tend to be located on plasmids. Analyses of selection on duplicates of different divergence classes suggest that most duplicate pairs are under strong purifying selection against amino acid change. The likelihood of duplicate retention varied among gene functional classes, and the pronounced differences between strains in the pool of retained recent duplicates likely reflects differences in the nutrient status and other characteristics of their respective environments. We conclude that most duplicates are quickly purged from *Acaryochloris* genomes and that those which are retained likely make important contributions to organism ecology by conferring fitness benefits via gene dosage effects.

INITIAL INSIGHTS FROM THE *NAEGLERIA FOWLERI* GENOME INITIATIVE

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The ‘‘Brain-Eating Amoeba’’ *Naegleria fowleri* is the causative agent of primary amoebic meningoencephalitis, a water-borne disease of the central nervous system. *N. fowleri* has a non-pathogenic, non-thermotolerant relative, *Naegleria gruberi*, for which the genome sequence has recently been determined. The *N. gruberi* genome sequence forms the perfect background to investigate the genetic basis of pathogenicity in *N. fowleri* through comparative analysis. We have produced a100X coverage of the *N. fowleri* nuclear genome using a combination of 454 pyro-sequencing and Illumina Hi-Seq technologies. We here report an initial meta-comparison between the ~50 KB mitochondrial genomes and a contig of nuclear genomic DNA of approximately equivalent size of the two *Naegleria* species. These show widely different patterns of conservation and degrees of synteny. Our initial meta-comparison lays the groundwork for a full-scale genomic comparison allowing eventual identification of molecular markers to be used for improved diagnosis, and putative genes involved in *N. fowleri* pathogenesis. Together these will greatly improve our understanding of this globally distributed amoeba and how it kills.

UTILIZING EVOLUTIONARY GENE NETWORKS TO UNCOVER GENOMIC CONSEQUENCES OF ADOPTING A PARASITIC LIFESTYLE

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Parasites are present throughout the tree of life and evidence suggests they have arisen independently numerous times. Much is known about the functions of parasites due to their biomedical implications, however, there is limited understanding on how organisms become parasitic. Identifying the genes, and gene families, that are involved in parasitism requires information from both parasitic and free living organisms within the same lineage. Oomycetes, specifically members of the Saprolegniaceae, present a unique opportunity for investigating the evolution of parasitism because this family contains members that are both free living and parasitic. We have sequenced the genomes of *Achlya hypogyna* (facultative parasite) & *Thraustotheca clavata* (free living), two Saprolegnian oomycetes. Along with examining the close relationships within the Saprolegniaceae, there also exist completed obligate parasite genomes in the more distantly related Pythiales and Peronosporales that will be combined in comparative analyses. Using these data, we present a method to elucidate the evolution of gene families related to a parasitic lifestyle, using evolutionary gene networks. EGNs are mathematically based graphs that can be used to visualize gene relationships that traditional phylogenetics would fail to resolve. Using this method we have identified unique gene family expansions, contractions and combinations that are potentially key to the evolution of parasitism within this group of organisms.

PERSISTENT AND WIDESPREAD ASSOCIATIONS OF APICOMPLEXANS (CHROMALVEOLATA) WITH CARIBBEAN REEF CORALS

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Apicomplexans are an important parasitic group, but little is known about those associated with many invertebrates, such as tropical reef-building scleractinian corals. Before resolving their potential influence on coral health, it is first necessary to further describe this enigmatic group and determine their prevalence among host species. To this end, three Caribbean scleractinian species (*Montastraea annularis, M. faveolata* and *Porites astreoides*) were sampled seasonally over 10 years (May 2000-present) from two reefs in the Florida Keys as well as over 6 years (May 2001–Nov 2005) for two Bahamian reefs. Utilizing PCR-based screening, these colonies exhibited chronic infection over the sampling duration. Furthermore, small subunit (18S) ribosomal DNA (rDNA) and internal transcribed spacer region 1 (ITS1) sequences derived from apicomplexans inhabiting other scleractinian and gorgonian colonies at these reefs indicate host specificity. For example, apicomplexan sequences derived from *M. annularis* exhibited high similarity within as well as between locations whereas substantial divergence was found among those infecting different coral genera on these reefs. Understanding the nature of this potentially parasitic symbiosis may offer new insight into the biology of invertebrate-protistan relationships.

SYNDINIAN SEX

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Syndinian dinoflagellates produce dinospores suggested to be part of the sexual cycle. Reports of pairing and apparent fusion of dinospores are open to interpretation, and the fate of presumptive zygotes remains unknown. Species of *Duboscquella* parasitize protists, with individual infections typically producing either highly motile macrospores, or weakly motile microspores. Study of a previously undescribed *Duboscquella* species from the tintinnid *Favella panamensis* revealed the formation of three spore types: large, spherical spores (females); large, egg-shaped spores (neuters), and small, bi-flagellate spores (males). Short sporocyte chains gave rise to non-motile female and neuter spores that slowly dispersed from the host lorica. Male spores were highly motile and formed from long, beaded sporocyte strands that exhibited serpentine movement. Male spores copulated with female spores, but not with neuter spores. Gamete fusion required 10–20 min and was accompanied by flagellar resorption and nuclear fusion. Successive division of zygotes yielded four daughter cells that closely resembled neuter spores. Male spores were short-lived (~1 day), while female spores, neuter spores, and zygote daughter cells persisted for 3–4 days. Infectivity of spore types is unknown.

NOVEL MICROSPORIDIAN SPECIES INFECTS THE FREE-LIVING MARINE NEMATODE *ODONTOPHORA RECTANGULA*

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This is the first report of a free-living marine nematode (*Odontophora rectangula*) infected with a microsporidian parasite. The 16S sequence of the new microsporidian indicates that it is closely related to the Encephalitozoon group, which includes human parasites. In *O. rectangula,* microsporidian infection prevalence appears high while virulence is low. Host examinations with fluorescent *in situ* hybridization (FISH) suggest that modes of infection differ remarkably between males and females. Infected males generally contain microsporidian spores concentrated in ''pockets'' that appear to be distributed randomly within their hypodermal and muscular tissues. Females appear to lack spores, while possessing predicted intracellular stages of the parasite restricted to their eggs. These results suggest that infection is transmitted vertically and that the immune system of males and females responds differently to microsporidian infection during development. The unique host and microsporidian interactions described here, the ability to maintain infection in the lab, and the close phylogenetic relationship of this microsporidian to its human-infecting relatives, could provide a rare opportunity to establish a model for the study of microsporidiosis.

ULTRASTRUCTURE AND MOLECULAR PHYLOGENY OF A MARINE ISOLATE OF *TRIMASTIX MARINA*

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The isolated free-living flagellate genus *Trimastix* is the closest relative of the well-known anaerobic symbiont group oxymonads, and thus is important for understanding the evolution of anaerobic mitochondrial organelles and the phylogeny of the supergroup Excavata. A marine isolate of *Trimastix marina* was studied for the first time combining light microscopic, electron-microscopic and molecular phylogenetic methods. The new isolate shows features seen in a previously studied freshwater isolate of *T. marina,* e.g. four flagella, thickened anterior flagellum, small, double-membrane bounded organelles without cristae. However, it also shows features distinct from freshwater *T. marina,* for example, the anterior basal body possesses cartwheels, the anterior microtubular root is small. Strikingly our molecular phylogenetic analyses never place marine *T. marina* with freshwater *T. marina,* or even specifically with other *Trimastix* species. Multigene (small subunit ribosomal RNA, beta-tubulin protein and heat shock protein 90) phylogenetic analyses place marine *T. marina* as the sister group of a clade composed by other *Trimastix* species and oxymonads. These results indicate a paraphyly of *Trimastix* as currently understood, and likely require a revision of the genus.

ULTRASTRUCTURE, DIVERSITY, AND PHYLOGENY OF COLPODELLIDS AND COLPONEMIDS, AND THE EVOLUTION OF ALVEOLATES

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Alveolates comprise a major eukaryotic phylum including a great diversity of ecologically, economically, and medically important species. Understanding alveolate evolution is important for addressing many intriguing questions, such as origins of parasitism in apicomplexans, evolutionary history of plastids and endosymbiosis and the unique organization of apicomplexan and dinoflagellate mitochondrial genomes. The biology, morphology, and ultrastructure of *Colpodella pseudoedax* (Mylnikov 2007), *C. unguis* (Patterson and Simpson 1996), *Colponema* aff. *loxodes* (Stein 1878), *C. edaphicum* (Mylnikov and Tikhonenkov 2007), and *C. marisrubri* (Mylnikov and Tikhonenkov 2009) were investigated. We obtained sequences of 18S ribosomal RNA genes of species and assess their evolutionary position based on combined evidence from ultrastructural, morphological and molecular data. To extend this data pool we are currently obtaining information from several other molecular markers and have launched transcriptomics projects on two colpodellid species. This data will be important in reconstructing alveolate phylogeny based on concatenated datasets of multiple genes, and providing insights into the evolution of unique cellular and genomic innovations, such as myzocytosis, the apical complex, the dinokaryon, splice leaders etc.

COMPREHENSIVE PHYLOGENETIC RECONSTRUCTION OF AMOEBOZOA BASED ON CONCATENATED ANALYSIS OF SSU-RDNA AND ACTIN GENES

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Evolutionary relationships within Amoebozoa have been controversial for two main reasons: (1) paucity of morphological characters in traditional surveys and (2) haphazard taxonomic sampling in modern molecular reconstructions. Here we provide a comprehensive phylogenetic reconstruction based on 139 taxa using molecular information from both SSU-rDNA and actin genes. We provide novel molecular data for 13 of these taxa, 12 of which had not been previously characterized. Our analyses show that many of the morphologically established lower-level relationships (defined here as relationships equivalent to Order level or below) are congruent with molecular data. Further, six new groups are hypothesized based on these analyses. However, most proposed higher-level relationships are not recovered, though there is insufficient power in these analyses to reject most hypotheses. Contrary to expectations from previous studies, the inclusion of environmental sequences does not significantly improve the Amoebozoa reconstruction. Finally, in an effort to facilitate future sampling of key amoebozoan taxa, we provide a novel methodology for genome amplification and cDNA extraction from single or a few cells; this method is culture-independent and allows both photodocumentation and extraction of multiple genes from natural samples.

MARINE GREGARINES

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Marine gregarines are a poorly understood group of obligate endoparasitic single-celled eukaryotes (protists) that infect marine invertebrates, and are a sister group to some of the most notorious pathogens of humans and other vertebrates (i.e. *Plasmodium* and *Cryptosporidium*). This study aims to sample the vast diversity of gregarines in the marine environment using light microscopy, scanning and transmission electron microscopy and molecular data (i.e. small-subunit rDNA) in order to make inferences about evolution within gregarines and, at a larger scale, the Apicomplexa. Using 18s SSU rDNA and high-resolution scanning electron micrographs, we examined 10 novel species of marine gregarines representing two lineages: Archigregarines and Eugregarines.

POST-TRANSCRIPTIONAL REGULATION OF NITROGEN ASSIMILATION IN MARINE DIATOMS

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The regulation of gene expression is a multifaceted and dynamic process. While coordinated patterns of gene transcription are well known, there is increasing evidence of coordinated post-transcriptional processes regulating gene expression in both prokaryotes and eukaryotes. Previous studies of marine diatoms demonstrated that transcripts encoding nitrogen transporters and assimilatory enzymes are sequestered in cells under N-starvation, regulated at the translational level, and destabilized in the presence of ammonium. These results suggest that post-transcriptional processes are important in the coordination and regulation of N-assimilation in marine diatoms. RNA-binding proteins (RBPs) play an important role in the post-transcriptional regulation of gene expression. Members of several RBP families are represented in the genomes of marine diatoms and the structure of these RBP families will be discussed. Electrophoretic mobility shift assays demonstrated that proteins bind to the 3'untranslated regions of *Skeletonema costatum* nitrate reductase and glutamine synthetase mRNA. RNA-protein binding profiles are currently being examined under various growth conditions, and in other diatom species, to further test the hypothesis that post-transcriptional processes are important in the regulation of N-assimilation in diatoms.

THE EFFECTS OF COPPER TOXICITY ON *PSEUDO-NITZSCHIA* SPP. PHYSIOLOGY AND DOMOIC ACID PRODUCTION

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Some species of the diatom genus *Pseudo-nitzschia* produce domoic acid, a phycotoxin that has been hypothesized to chelate Cu and reduce Cu toxicity to the cells. To better characterize the effect of Cu on Pseudo-nitzschia, a toxic strain (*P. multiseries*) and a non-toxic strain (*P. delicatissima*) were exposed to Cu(II) for 96 h (50 μg/L for *P. delicatissima*, 50, 100 and 150 μg/L for *P. multiseries*). Each day, physiological measurements were performed on *Pseudo-nitzschia* cells to determine the concentration of cells, lipid content, chlorophyll, domoic acid (DA) content, enzymatic activity, photosynthetic efficiency, O_2 consumption of cells, and the percentage of dead algal cells. After 48 h of Cu exposure, *P. delicatissima* mortality increased drastically whereas *P. multiseries* survival was unchanged in comparison to unexposed cells. Cellular enzymatic activity, chlorophyll content and lipid concentration significantly increased upon Cu exposure in comparison to unexposed cells (24 h for *P. delicatissima*, up to 96 h for *P. multiseries*). DA concentrations in *P. multiseries* were not modified by Cu exposure. Results suggested that cells of *P. delicatissima* are much more sensitive to Cu than *P. multiseries.*

COMPARATIVE GENOMICS OF PROTISTS REVEALS EVOLUTION AND DIVERSITY OF THE ARF GAP PROTEIN FAMILY

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The majority of models concerning the mechanism of membrane-trafficking have been developed primarily through studies of animals and yeast. Although these are well-characterized model systems, they are not necessarily representative of the full diversity of eukaryotes, especially with respect to protists. Comparative genomic analyses with representatives from major eukaryotic lineages have revealed an ancient origin for many membrane-trafficking families. Herein we describe a comparative genomic analysis of the Arf GAP family of proteins; key regulators of membrane-trafficking. We searched 34 genomes for the ten Arf GAP sub-families, as defined in humans, and found that seven are conserved across the diversity of eukaryotes and were present in the eukaryotic ancestor. The other three are exclusive to holozoa. Phylogenetic analysis of individual sub-families revealed two patterns of evolution specific to the Holozoa. Analysis of domain conservation revealed only a small number of domains present in the ancestral form of these proteins and in protists, in contrast to the many domains identified for the holozoa.

FROM SPEARS TO ARMOR: EVIDENCE THAT STREPTOPHYTE WALL CELLULOSE ORIGINATED FROM PROTISTAN EXTRUSOMES

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Cellulose is an essential component of the cell walls of land plants and diverse algae, conferring protection and structural support, and having diverse technological applications. In walled streptophytes, cellulose microfibrils having distinctive physical characteristics are spun out from rosette protein complexes. How streptophyte rosettes originated is unknown, because the cellulose synthesizing complexes of bacteria and other algal lineages are linear to rectangular in shape. Noting that: (1) similar rosettes occur within extrusome envelopes of cryptomonads, identified by our molecular phylogenetic analyses as streptophyte relatives; (2) extrusomes similar to those of cryptomonads occur in related heterotrophic protists and wall-less prasinophytes; and (3) extrusomes display glycosyl residues, we hypothesized that the cellulosic component of streptophyte cell walls may be derived from extrusomes ancestrally present prior to endosymbiotic acquisition of the streptophyte plastid. Our hypothesis is supported by observations of bifrefringence and calcofluor staining typical for beta-glucans and consistent with extrusome distributions in diverse protists related to the streptophyte lineage. Genetic changes leading to functional modification of glycosyltransferases and their deployment into the plasmalemma likely occurred prior to the divergence of cellulose-walled streptophytes.

BREVETOXIN INTERACTION WITH VOLTAGE-ACTIVATED IONIC CURRENTS OF A MARINE DIATOM

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The HAB species, *Karenia brevis*, produces brevetoxins (PbTx) which bind to voltage-gated sodium channels (VGSCs) in nerve cells altering gating kinetics and causing membrane hyperexcitability. Recently discovered animal-like VGSCs in marine diatoms and other chromalveolates could be an ecologically relevant target for interaction. The effects of PbTX-3 were therefore examined on the VGSCs of the diatom *Odontella sinensis* using single electrode current and voltage clamp. Current clamp experiments showed that 1 μM PbTx-3 had no effect on membrane potential. Nevertheless under voltage clamp, PbTx-3 inhibited the evoked peak Na+ current by 33% with a positive shift in the reversal potential. No change in activation kinetics was observed, although a 4 mV positive shift in voltage-dependent inactivation suggests a slight change in kinetics from the open to close protein conformation. Results indicate that brevetoxins significantly affect permeation of the VGSCs while exhibiting limited impacts on channel kinetics. This unique interaction with diatom VGSCs is unlike those documented in animal systems but suggests that cellular signaling in some non-toxic phytoplankton could be affected by the presence of HAB secondary metabolites.

THE EFFECTS OF NITROGEN SOURCE ON THE PHYSIOLOGY AND PRIMARY PRODUCTIVITY OF THE COCCOLITHOPHORID *EMILIANIA HUXLEYI*

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A calcifying strain of *Emiliania huxleyi* (CCMP1516) was cultured under NO^{3-} and NH^{4+} as the major nitrogen sources at a light intensity of 300 μmol photon/m^2/s and temperature of 17 °C on a 18:6 light:dark cycle, in order to assess the effects of N source on photosynthetic physiology. Growth rate, cell volume and cellular chlorophyll a, were unaffected by N source, but significant differences were observed in the photosynthesis-light relationship, and in light-dependent O_2 consumption. Fast repetition rate fluorescence measurements were used to assess changes in photosystem II (PSII) operating efficiency. Both light-saturated and light-limited oxygen production were significantly reduced (~60%) in NH^{4+}, while O_2 uptake was increased resulting in significantly lower net production. Photosynthetic electron transfer rates showed inhibition at high irradiances in NO^{3-}, not observed for NH^{4+} cultures. There were no substantial changes in non-photochemical quenching, or energy utilization and efficiency of PSII between the two treatments. These results indicate that nitrogen source does not substantially affect PSII, but considerably reduces gross and net oxygen.

A NOVEL MECHANISM TO EXPLAIN CONTRACTION AND RELAXATION IN THE CILIATED PROTIST, *VORTICELLA CONVALLARIA*

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In, *Vorticella convallaria*, a mechanical stimulation results in a calcium-induced rapid contraction of the spasmoneme. Ultrastructural studies revealed two distinguishable regions within this organelle: a ''fibrillar mass'' composed of longitudinally oriented 2–5 nm fibers and ''membrane-bounded tubules'' that are evenly distributed throughout the fibrillar mass. The membrane-bounded tubules contain calcium suggesting a calcium storage/release function. Interestingly, these tubules are also filled with 2–5 nm fibers whose function is yet undetermined. We have developed a model that explains both the role of tubules as a calcium storage/release compartment and the role of centrin fibers within these compartments. Upon receipt of a signal, the tubules release calcium to the fibrillar mass and this change in calcium concentration causes disassociation of self-assembled centrin. Within the fibrillar mass, calcium binds to centrin/spasmin molecules that are tightly associated with SFI1p (a centrin-binding protein). This leads to coiling and shortening of the stalk. During stalk re-extension, calcium, released from centrin/SFI1p complexes, is pumped back into the tubules allowing self-assembly of centrin and restoring rigidity of tubular compartments which assists in the extension of the spasmoneme.

THE MARINE DINOFLAGELLATE GENUS *DINOPHYSIS* IS ON THE WAY TO GAINING PLASTIDS

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The marine dinoflagellate genus *Dinophysis* is an intriguing organism for investigating plastid evolution. To survive, the photosynthetic *Dinophysis* spp. must feed on the plastidic ciliate *Mesodinium rubrum* (= *Myrionecta rubra*), itself a consumer of cryptophytes. While photosynthetic *Dinophysis* species are well known to contain plastids of cryptophyte origin, whether the plastids are permanent or periodically derived kleptoplastids (stolen plastids) has not been confirmed yet. There has been an obvious contradiction between molecular and ultrastructural data for the status of the plastid. Phylogenetic studies comparing plastid gene sequences of the three organisms support the kleptoplastid hypothesis, mostly based on the result of genetically identical plastid gene sequences among them. However, TEM studies support permanent plastid hypothesis, based on ultrastructure (e.g. plastid surrounded by two membranes, terminal position of the pyrenoid, stellate compound structure, and the lack of nucleomorph) different from those in prey. Why is so different between the two and how can we explain this difference? We addressed this issue here using the established *D. caudata* culture as a model organism, single-cell TEM technique, and time-lapse video microscopy. The results will be discussed in the context of the status of *Dinophysis* plastids.

A PROTON CONDUCTANCE IN COCCOLITHOPHORES IS AN INTEGRAL COMPONENT OF PH HOMEOSTASIS DURING CALCIFICATION

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In most marine calcifiers, precipitation of calcite occurs extracellularly. However, in the unicellular heterococcolith bearing coccolithophores, calcification occurs in a specialized intracellular Golgi-derived coccolith vacuole (CV). The completed coccoliths are rapidly secreted onto the cell surface forming the outer coccosphere. At rates of up to one coccolith per hour, intracellular calcification requires rapid ion transport to the CV as well as intracellular homeostasis of calcium and protons. Significantly, 1 mol of protons are generated for every 1 mol calcite precipitated, presenting a large potential for intracellular metabolic acidosis. Effective regulation of intracellular pH is therefore essential. Biophysical analysis of *Coccolithus pelagicus* shows they exhibit a novel voltage activated proton conductance and also possess genes encoding voltage activated proton channels. The functional properties of the native and heterologously expressed proton channels bear a striking resemblance to proton conductances previously only described in animal cells that undergo metabolic acidosis. We propose that the coccolithophore proton conductance is an integral component of the pH homeostatic mechanism during calcification. Moreover, our data suggest an early evolution of this eukaryote pH regulatory mechanism.

DIVERSITY IN IRON-LIMITED DIATOMS HAS VARIED EFFECTS ON SILICON CYCLING

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Iron limitation is known to affect diatom chemical composition and morphology. Minor changes in the resulting elemental stoichiometry affect biogeochemical cycles, but predicting the impact of these changes is confounded by the diversity of diatom species. Using a combination of molecular and microscopy techniques, we analyzed lab isolates and field populations experiencing iron-limitation to determine the potential ways different species influence biogeochemical cycles. Of the four cell wall related genes assessed, several are part of multi-copy gene families that have diverged within species and are regulated differently among species. One of the genes identified was a silicon transporter in *Pseudo-nitzschia* that is regulated by iron limitation. Homologous silicon transporters were detected in an iron-limited community in the NE subarctic Pacific and appear to be regulated similarly to lab isolates, as shown by a metatranscriptomics. Quantification of new silica precipitation also indicates that *Pseudo-nitzschia* populations dominated silicon cycling, even when it was not the most abundant species. A combination of molecular and microscopy techniques suggests that abundance, size, and growth rate may all affect biogeochemical cycles differently.

NUCLEUS- AND NUCLEOMORPH-TARGETED HISTONE PROTEINS IN A CHLORARACHNIOPHYTE ALGA

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The plastid of chlorarachniophyte algae possesses a relict nucleus, referred to as the nucleomorph, derived from a green algal endosymbiont, which is located in the periplastidal compartment (PPC). The nucleomorph genome of a chlorarachniophyte, *Bigelowiella natans*, encodes several plastid-targeted proteins and hundreds of housekeeping proteins, but it lacks many fundamental genes to maintain itself. Here, we present the first two host nucleus-encoded genes for proteins targeted into the nucleomorph, histone H2A and H2B. These two histones were predicted to be derived from the symbiont based on phylogenetic analyses, and both genes encode N-terminal extensions resembling PPC targeting signals. Using GFP fusion proteins expressed in transformed cells, we demonstrated that the putative symbiont H2A and H2B were targeted into the nucleomorph. Furthermore, we have developed a method to temporarily synchronize *B. natans* cells, and confirmed that the expression of these histone genes is controlled during the cell cycle. Our findings provide the first evidence of how the nucleomorph may be regulated by host-encoded gene products.

DATA MINING AND LOCUS DISCOVERY METHODS IN THE BROWN ALGAE (PHAEOPHYCEAE)

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Worldwide, the brown algae are one of the most species rich and ecologically critical groups of marine primary producers. However, despite their importance, understanding their evolution or phylogenetics remains elusive. One of the hindrances to advancing our understanding is the lack of adequate genes to resolve the brown algal tree. The goal of our project is use a bioinformatic data mining approach to develop nuclear markers for a comprehensive multigene brown algal phylogeny with extensive taxon sampling. To accomplish this goal, we created a genetic database that included all sequence data (published and novel transcriptome data we generated) for brown algae and related outgroups. We clustered homologous sequences and found all loci that were shared among most members of these lineages. Resulting gene alignments and trees were evaluated and candidate loci were identified. Primers for positive loci were constructed and screened against seven brown algal orders spanning the breadth of the class

and sequenced to further evaluate their utility. To date, 70 loci have been screened and 12 positive loci (> half of our target of 20) have been identified.

A MORPHOLOGICAL CHARACTERIZATION AND MULTIGENE ANALYSIS OF *DRACONELLA HESITOSOLIS* NOV. GEN. NOV. SP.

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In this investigation, morphological and multigene analyses are used to identify and confirm the phylogenetic placement of the novel vampyrellid amoebae, *Draconella hesitosolis* nov. gen. nov. spp. Detailed observations of a key life cycle form among other physical traits and behaviors observed using scanning electron, light, and fluorescence microscopy distinguish this isolate as a new genus and species of vampyrellid organism. While *Draconella hesitosolis* nov. gen. nov. spp. exhibits many similarities to other vampyrellid organisms, particularly to *Leptophyrs*, which is also densely vacuolated, has distinct endo- and ectoplasmic regions, and engulfs food rather than punctures the cell walls of its prey like members of the genera *Vampyrella*, *Gobiella*, and *Lateromyxa*; it is distinguished from existing vampyrellid genera based on a combination of features including: a floating form, multiple nuclei, anastomozing pseudopodia, and low numbers of offspring. In addition, analyses of four concatenated genes (SSU-rDNA, actin, hsp70, and hsp90) within a comprehensive dataset place the isolate within the major eukaryotic group ''Rhizaria'', sister to the available vampyrellid sequences. Support for this assessment comes from single-gene SSU-rDNA geneologies, as well as the presence of an amino acid insertion in the ubiquitin gene, which is shared by another well supported group of organisms known as Formaninifera also within ''Rhizaria''.

CALOTHRIX OR *SCYTONEMATOPSIS*? A NEW SPECIES OF CYANOBACTERIA THAT DEFIES GENERIC PLACEMENT

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We recently isolated a novel cyanobacterial strain from a damp fallen log in Oahu, Hawaii. The species fits the morphological description of *Scytonematopsis* in that it possesses isopolar filaments with double false-branching and tapering trichomes. Isopolar taxa with double false branching are placed in the Scytonemataceae, while heteropolar taxa with single false branching are placed in the Microchaetaceae. Unbranched tapering filaments are in the Rivulariaceae. Our taxon would be a new species if circumscribed within *Scytonematopsis*. However, phylogenetic analysis based on 16S rRNA gene sequence data shows this strain to be in *Calothrix*, even though it does not fit either the circumscription of *Calothrix* or the Rivulariaceae. Secondary structure of the 16S–23S ITS region of the new taxon is highly similar to the secondary structure of other *Calothrix* species, but very different from the secondary structure of the 16S–23S ITS region of *Scytonematopsis contorta*, the only species of *Scytonematopsis* that has been sequenced. This species challenges our conception of these genera and families, and cannot be placed in any existing genus unless either morphology or sequence data are ignored.

DIVERSITY OF THE SYNDINIAN GENUS *DUBOSCQUELLA*

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Syndinian dinoflagellates are marine endoparasites with hosts ranging from crustaceans to radiolarians and even other dinoflagellates. Ribosomal sequences attributed to syndinians fall into two unequal groups: tens of sequences from described genera versus thousands of sequences from environmental clone libraries. The syndinian genus *Duboscquella* was first described from tintinnid ciliate hosts and contains eight described species, five from tintinnids. In the current study *Duboscquella* was found in eight different tintinnids, and infected hosts from field samples were used for single cell PCR. Naïve host sequences were also determined using hosts from the same samples. Ribosomal host and parasite phylogenies did not indicate strong patterns of coevolution. The sequence variation within and between individual infections was also assessed using replicate single cell PCR in all cases, combined with cloning for three of the parasites. Overall there was little overlap between the environmental clone library dataset and the emerging clade of sequences attributed to *Duboscquella*.

THE CONFUSING STATE OF CORALLINE (CORALLINALES, RHODOPHYTA) TAXONOMY IN THE NORTHEAST PACIFIC: LOCAL AND GLOBAL IMPLICATIONS

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Molecular sequencing of northeast Pacific field-collected corallines has revealed more species than available names. But what is the correct specific epithet for each species, and to what genus does each species belong? Because of the morphological simplicity and/or plasticity of both articulated and crustose corallines, and because we don't know which morphological/anatomical characters are phylogenetically informative, we have had difficulty answering these fundamental questions. I will show that only by sequencing type specimens can the first question be answered, and only by including generitype species in phylogenetic analyses can the second question be resolved. Examples of both methods will be shown to resolve northeast Pacific species in the genera *Mesophyllum* and *Bossiella*, whereas problems remain with northeast Pacific species of *Pseudolithophyllum*. Molecular sequence data are resolving coralline species into genera more reliably than currently used morphological/anatomical characters. The application of species names far removed from their type localities, particularly for crustose corallines, needs to be tested with methods that do not rely on characters of morphology/anatomy.

A SYSTEMATIC STUDY OF *TOLYPELLA* A. BR. (CHARALES, CHAROPHYTA): RECENT ANALYSES BASED ON PLASTID SEQUENCE DATA.

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The Characeae contain two tribes with six genera: tribe Chareae, which includes *Chara*, *Lamprothamnium*, *Lychnothamnus* and *Nitellopsis*; and tribe Nitelleae, which includes *Tolypella* and *Nitella*. The Characeae exhibit a broad range of morphological diversity and in the most comprehensive taxonomic treatment over 400 species were consolidated into 81 broadly defined species. Within this treatment, 16 *Tolypella* species were subsumed under two species (*T. intricata* and *T. nidifica*) in two sections, Rothia and Tolypella, respectively. It was further suggested that *Tolypella* might be a derived group within *Nitella*. Currently, there are no comprehensive molecular phylogenetic studies of *Tolypella*. Instead, phylogenetic studies of Characeae and closely related taxa have sampled between one and three of the 16 described *Tolypella* species to serve as placeholders or outgroup taxa. In our ongoing investigation of species diversity in *Tolypella*, plastid sequence data were assembled from field-collected and herbarium specimens. Phylogenetic analyses of plastid sequence data supported monophyly of *Tolypella* and of sections Rothia and Tolypella. Also, several clades were identified which suggests greater species diversity than currently recognized.

THE LOCALIZATION AND PHYLOGENY OF ROACH-INFECTING *ENTAMOEBA*

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While *E. histolytica* has been well-studied for its role in human pathogenesis, the biodiversity of other *Entamoeba* remains poorly understood. Preliminary data of small subunit (ssu) rRNA gene sequences from insect guts revealed a novel clade of insect-infecting *Entamoeba*, with a diversity rivaling that of all other *Entamoeba* combined. In the current study we aimed to flesh out this diversity by collecting *Entamoeba* ssu rRNA gene sequences from different organs of the cockroaches *Periplaneta americana*, *Blatta germanica* and *Blaberus giganteus*. Most of the detected variants fell within the insect-infecting clade. The few that did not were variants of *E. moshkovskii*, a potential human pathogen, suggesting a role for cockroaches as disease vectors. Our hypothesis that *Entamoeba* invades non-gut tissues in insects as it does in vertebrates was supported by the detection of *Entamoeba* in these regions in addition to being found throughout the alimentary canal. Patterns of host specificity and differential localization of variants were not apparent, though the sampling of more host specimens in the future will help resolve this.

MOLECULAR SYSTEMATICS OF *COMPSOPOGON* (RHODOPHYTA, COMPSOGONALES) IN BRAZIL

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Species level taxonomy and phylogenetic relationship among 10 populations of *Compsopogon* in Brazil were investigated using two molecular markers - rbcL gene and cox1 barcode region. Analyses based on rbcL sequences revealed a tree with one large clade with high support (100% bootstrap, 1.00 posterior probability), representing the family Compsopogonaceae and the single genus *Compsopogon.* All Brazilian sequences and nine from other regions of the world (six from Genbank and three unpublished sequences) were included in this clade. Sequence divergence among *Compsopogon* was relatively low: 0–8 bp, 0–0.8%. Analyses based on cox1 sequences also revealed a low sequence divergence among all Brazilian samples: 0–7 bp, 0–1.1%. The two previously recognized species for Brazil on a morphological basis, *C. coeruleus* (cortex with regular polygonal cells) and *C. leptoclados* (cortex with rhizoidal cortical cells), were not supported by the molecular data. Results indicate that all sequences represent a single species – *C. coeruleus* (the type species of the genus), and the family Compsopogonaceae is monotypic. An ongoing investigation with a more representative world sampling will evaluate this taxonomic scheme.

MOLECULAR TOOLS EXPOSE MISIDENTIFIED AND NOVEL TAXA AMONG BERMUDIAN RHODOPHYTA

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The use of molecular-assisted alpha taxonomy (MAAT) has become increasingly widespread for red algae (Rhodophyta), whose variable morphology has historically made species-level identification challenging. We applied these methods to the marine flora of Bermuda, an isolated archipelago located at the interface of tropical and warm temperate zones. Based on work done over the last decade, it is clear that a significant number of Bermuda's algal species have been misidentified, and the level of endemism in these islands may be underestimated. Using multi-gene molecular analysis, we have uncovered 46 species currently classified as 19 biological entities, at least 24 of which may be novel taxa. A single taxon, *Centroceras clavulatum*, is currently recognized for Bermuda, yet five were revealed by MAAT analysis (two potentially novel species). The *Laurencia obtusa* complex is under investigation because it forms three distinct species in phylogenetic analysis, none of which include the type locality. Additionally, the genus *Peyssonnelia* is resolved into a minimum of six clades in our analyses, four of which are likely novel.

PHYLOGENY OF THE EUSTIGMATOPHYCEAE

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The Eustigmatophyceae as currently defined comprise only a few genera and species, primarily from soil and marine environments. However, recent work with freshwater isolates has revealed a wealth of diversity in the class. Some of this new diversity may be organisms that were previously referred to the related class Xanthophyceae, but many isolates that may represent new genera and species are ''little green balls'' with few distinguishing features apparent by light microscopy. Results of phylogenetic analyses of plastid rbcL and nuclear 18S rDNA sequences from these isolates and authentic strains of named Eustigmatophyceae indicate a need to revise and expand the taxonomy of the class. We have detected several new lineages among the freshwater isolates, including possible new genera, families and orders. On the other hand, our results suggest that the currently accepted genera *Vischeria*, *Eustigmatos* and possibly *Chloridella* should be merged into a single genus. We are presently expanding the characterization of these isolates with the goal of finding features of the life histories or zoospore characteristics that will provide additional insights on the relationships among the Eustigmatophyceae isolates.

EVOLUTION OF THE HETEROKONT ALGAE AS SEEN FROM ORGANELLAR GENOMES

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The heterokonts (or stramenopiles) encompass diverse and ecologically successful lineages of chlorophyll c-containing algae, including diatoms, pelagophytes, and chrysophytes. Like haptophytes, photosynthetic heterokonts are secondary endosymbionts of the red lineage. However, despite their morphological diversity and ecological importance, evolutionary relationships among the various classes remain largely unresolved. We have combined a fosmid cloning approach with the illumina short-read sequencing platform to target the organellar genomes. Here we use both plastid and mitochondrial genome sequences from 21 taxa representing 10 major classes to reconstruct the phylogeny of the heterokonts. The organellar genome sequences provide insight into plastid to nuclear gene transfer in secondary endosymbionts and offer additional loci for diversity studies in the field. A more accurate phylogeny of the heterokont lineage has implications for timescales of evolution of the major classes and suggests priorities for future candidates for whole genome sequencing.

THE DEEPER-LEVEL PHYLOGENY AND EVOLUTION OF HETEROLOBOSEA: A MAJOR GROUP OF PROTISTS

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Heterolobosea (Page and Blanton 1985) is a major group of protozoa that has increased substantially in known diversity since its proposal. In particular, the amoebae and amoeboflagellates that were originally included are now affiliated to a range of 'flagellates' as well. The major phylogenetic relationships within Heterolobosea (*sensu lato*) are not well understood, and the evolutionary history and deep-level systematics of the group is debated. We have examined the molecular phylogeny of Heterolobosea, with two foci – firstly, multiple lineages of the genetically diverse flagellate 'species' *Percolomonas cosmopolitus*, which is related to the multiflagellated *Stephanopogon* and, secondly, some newly cultured deep-branching lineages. We find that *P. cosmopolitus* is actually monophyletic: Earlier results suggesting that *P. cosmopolitus* is the paraphyletic stem for *Stephanopogon* seem to have been analysis artifacts. Whereas an origin of amoebae within Heterolobosea (*sensu lato*) has been suggested recently, we find that the deepest branches within this group include amoebae. It is probable, therefore, that amoebae were an ancestral feature of the entire Heterolobosea (*sensu lato*) clade, and that Heterolobosea is the appropriate name for this group.

EVOLUTION OF PHENOTYPIC DIVERSITY IN PICOCYANOBACTERIA FROM THE GULF OF MEXICO

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Within marine *Synechococcus*, phycoerythrin (PE) spectral phenotype is an ecologically important character associated with distinct optical regimes. Evidence has revealed the adaptive evolution of this phenotype independent of the core genome. However, PE is found throughout the picocyanobacterial radiation, and the ecological and evolutionary processes underlying PE variation in this broader context remain unclear. Here we report on the occurrence of a wide range of spectral phenotypes within a single sample from the Gulf of Mexico and evaluate the evolutionary history of the genes that underlie this diversity. Combinations of molecular evolutionary and physiological approaches indicate PE genes associated with distinct phenotypes form strongly supported monophyletic clades with directional evolution generally adapting towards bluer water. However some evidence also suggests that PE best suited to greener water evolved via gene loss in marine *Synechococcus*, with the presence of this phenotype in marine *Cyanobium* best explained by horizontal gene transfer. Our field data show that picocyanobacterial communities maintain genetic and phenotypic diversity sufficient for both physiological adaptation and natural selection to function as mechanisms for observed community-level changes in spectral phenotype.

IDENTIFYING LATIN AMERICAN *CHARA* (CHARALES: CHAROPHYTA) USING DNA SEQUENCES

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The New York Botanical Garden Herbarium (NY) houses more than 500 specimens of *Chara* collected from Latin America. These specimens were catalogued and their collection information and taxonomic annotations were made available online (sciweb.nybg.org/science2/hcol/chara/index.asp).

DNA was extracted from 339 specimens and attempts were made to amplify and sequence the plastid genes *atpB* and *rbcL*. Resulting sequences were incorporated into a larger data set that included numerous *Chara* species from around the world. Thirty-four percent (116) of the specimens were successfully sequenced to some extent. Sixty-six percent (76) of those specimens were analyzed phylogentically. Of those specimens, the molecular data were consistent with the original determination for ~49% (37), and inconsistent for ~43% (33). Six previously unidentified specimens were identified to species with these data. Due to incomplete sequence data, we were unable to identify ~36% (42) of the specimens to species using phylogenetic methods. However, we were able to place these individuals into a broader phylogenetic context (e.g. sections). These data contribute to our understanding of the occurrence and biogeographic patterns of *Chara* in Latin America.

HOST-SPECIALIST LINEAGES DOMINATE THE ADAPTIVE RADIATIONS OF ENDOSYMBIOTIC DINOFLAGELLATES (*SYMBIODINIUM*)

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Adaptive radiations have occurred throughout the history of life. Many of these bursts of diversification occur when populations differentiate from a common ancestor through ecological specialization. Clade C *Symbiodinium* is the most diverse and ecologically dominant group of endosymbionts (zooxanthellae) found in corals. In the Caribbean, phylogenetic reconstructions based on rapidly-evolving non-coding sequence data from plastid psbA mini-circle, and supported by nuclear ribosomal DNA spacer regions, identified numerous independent monophyletic lineages, each comprising haplotypes associated with specific host taxa. Portions of these lineages are further differentiated by geographic region. The level of genetic divergence between *Symbiodinium* clade C lineages is indicative of high species diversity according to ecological and phylogenetic species concepts. When compared to counterparts from the Indo-Pacific, this clade appears to have undergone a major diversification from a common ancestor beginning in the late Miocene-early Pliocene as the planet grew cooler and CO_2 levels reached their lowest levels in 300 MY. These lineages and the specificity they display therefore appear long-lived and most are probably incapable of associating with different hosts in response to climate warming.

HIGH-FREQUENCY WATER QUALITY MONITORING IN THE CENTRAL INDIAN RIVER LAGOON, FLORIDA

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Water quality in the Indian River Lagoon (IRL) has changed significantly over the past eight decades due to watershed alteration and land drainage patterns. High-frequency water quality monitoring, along a perceived water quality and seagrass gradient, has been underway since May 2005 in the IRL between northern Vero Beach and Fort Pierce. Temperature, salinity, dissolved oxygen, pH, turbidity, and chlorophyll a are being continuously monitored with datasonde multiprobes; color, suspended solids, nutrients, and light attenuation coefficients (K) are measured weekly. Overall, from north to south, salinity increases, while turbidity, color, suspended solids, and chlorophyll a (all attenuators of light) decrease, as do nutrients and K. The magnitude of most of these patterns varies considerably from year to year due to interannual variability in precipitation and freshwater discharge into the lagoon. Results to date demonstrate the tremendous climate-related interannual variability in water quality in the IRL and can be used in models of expected positive improvements in estuarine health following the reduction of freshwater inputs, which are recognized as the most significant human impacts on this estuary.

SEPARATION OF EUKARYOTIC BACTERIVORE DNA FROM ENVIRONMENTAL SAMPLES

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We present a novel method to isolate the DNA of bacterivores from environmental samples. Live bacteria took up a thymidine analog, bromodeoxyuridine

(BrdU), and incorporated it into their DNA, verified by dot-blotting. These labeled bacteria were then incubated with natural water samples from marine and lake environments. After incubation, whole DNA was extracted. Labeled DNA from these extracts was isolated using immunoprecipition (IP) with anti-BrdU antibodies. Selectivity of the IP was verified by qPCR of labeled and unlabeled template DNA. Eukaryotes from the environmental samples were successfully labeled with BrdU. This method separates DNA from environmental samples by functional category. Like DNA stable isotope probing (DNA-SIP), it allows for downstream genetic analyses (such as T-RFLP, DGGE, cloning, and next-gen sequencing) that answer questions beyond whole community characterization. By isolating DNA from bacterivorous microbial eukaryotes, this method will help expand our understanding of trophic interactions in the microbial biosphere and of the relationship between biodiversity and ecosystem function.

DNA BARCODING OF PROTISTS IN SOILS: A CASE STUDY USING ARCELLINID TESTATE AMOEBAE (AMOEBOZOA)

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Species identification in protists is traditionally based on morphological features. However, the taxonomic validity of characters used to recognize morphospecies remains unclear, which is problematic for addressing fundamental questions in protist soil ecology, biogeography and evolutionary history. In order to evaluate the utility of the Cytochrome Oxidase Subunit 1 (COI) marker for more accurately identifying species of protists, we focused on arcellinid testate amoebae as a case study. Arcellinid testate amoebae are key players in soil microbial food webs and important contributors to nutrient cycling. We obtained 100 COI sequences belonging to 25 morphospecies isolated from soil litter and moss habitats. This approach allowed us to discriminate all of the morphospecies and revealed several potential cryptic species. The study showed that COI is a good candidate for DNA barcoding species of arcellinid testate amoebae. However, in order to more broadly use this marker in soil molecular ecology, suitable primers need to be developed for other groups of arcellinid testate amoebae.

HOW MANY KINDS OF CILIATES IN A COASTAL PLANKTON SAMPLE? COMPARISON OF CLONE LIBRARY AND PYROSEQUENCING ESTIMATES

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We designed primers for the dominant clade of coastal planktonic ciliates (oligotrichs plus choreotrichs from the class Spirotrichea) and used them to construct SSU clone libraries from samples collected in Long Island Sound, an estuary in the Northwest Atlantic. Stations were selected to provide a range in environmental variability, including the Connecticut River and its outflow plume, the stratified open Sound, and shallow nearshore waters. Clone libraries produced the familiar pattern of a few dominant operational taxonomic units (OTUs) and many rare ones, without evidence of saturation. Even so, the total number of observed OTUs was equal to or greater than the number of previously observed morphospecies, based on microscope counts. Pyrosequencing (454 technology) produced > 30,000 sequences from the same samples. When contigs were assembled at 97% similarity and single-sequence contigs were eliminated, results were broadly concordant with the clone libraries, except that about twice as many OTUs were observed, and the 454 data was dominated by choreotrichs. In both sequence collections, only a few OTUs could be identified by BLAST on GenBank, indicating that most species are yet to be sequenced.

PSEUDOPOD: PROTISTS REACH OUT

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With research centers, funding agencies and taxpayers placing increasing emphasis on the communication of science to the public, how can scientific communication be expanded beyond traditional publication in peer-reviewed literature? Most researchers agree that a public dialog is important, but insufficient knowledge of outreach-friendly communication methods and lack of time may hamper individual efforts. We will discuss how seemingly unrelated resources (blogging platforms, media contacts, biological databases and computer graphics) can be used to present a cross-platform, readily accessible window into modern protist research. These tools not only improve communication with the public, but can also improve scientific productivity and enhance collaboration. Emphasis will be placed on tools that are within the reach of single research groups or very small consortia.

CRYPTIC SPECIATION WITHIN *BATRACHOSPERMUM* SECTION HELMINTHOIDEA (BATRACHOSPERMALES, RHODOPHYTA) REVEALED BY MULTIGENE MOLECULAR ANALYSIS

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Previous research, using the rbcL gene and to a lesser extent SSU sequence data, has placed the freshwater red alga, *Batrachospermum boryanum* Sirodot, in a well-supported clade with *B. confusum*, *B. confusum* forma *anatinum*, *B. heterocorticum*, and *B. involutum*. Furthermore, all of these taxa share the morphological synapomorphy of heterocortication. More than 165 specimens have been collected from a total of 51 locations; 33 in the USA, 4 in Great Britain, nine in Poland, two in Lithuania, and three in Finland. Using rbcL and cox1 sequence data, the relationships among these specimens were analyzed. Results of maximum likelihood and Bayesian analyses showed *B. boryanum* to be paraphyletic within the clade of heterocorticated taxa. From these preliminary data, there appear to be seven genetically distinct heterocorticated species, but more may be delineated with the addition of new specimens. The clades from the molecular data are not geographically structured and many of these taxa appear to overlap in their ranges. Future research will focus on type specimens of these species and linking them to these genetic data.

DIVERSITY AND DISTRIBUTION OF NANO- AND PICOEUKARYOTES IN THE SOUTHERN OCEAN AS REVEALED BY ENVIRONMENTAL SEQUENCING AND MICROSCOPY

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In this study we explored the diversity and distribution of planktonic nano- and picoeukaryotes in Antarctic waters by combining 454-pyrosequencing, clone libraries and electron microscopy. Water samples were collected and physico-chemical parameters measured during summer 2008 with the Norwegian R/V G.O. Sars in the Atlantic sector of the Southern Ocean, at stations along a transect (15°E) between 45–67°S. Ribosomal DNA of plankton in the size range 0.8–35μm was amplified with universal eukaryotic and haptophyte specific primers followed by pyro- and Sanger sequencing. Most rDNA sequences could not be assigned to a cultured and sequenced species (at $\geq 99.3\%$ identity level) indicating that a large fraction of the plankton community remains to be genetically characterized. Electron microscopy revealed some of this diversity, but more cultured strains are needed as reference for a precise identification. The taxonomic groups represented with highest number of clones/reads were dinoflagellates, stramenopiles and haptophytes. The species composition was significantly different at the northernmost station compared to further south, suggesting that nano- and picoeukaryotes species composition and distribution may serve as indicators of climate change in polar waters.

BACTERIAL COMMUNITY STRUCTURE IN *PORPHYRA UMBILICALIS* FROM THE MAINE SHORE IN AUTUMN AND WINTER ASSESSED THROUGH 16S RDNA SEQUENCING

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Seasonal variations in the bacterial community of *Porphyra umbilicalis* from the coast of Maine were assessed by performing 16S ribosomal DNA (rDNA) sequence analysis. Five blades per season were collected randomly along a transect at Schoodic Point in both fall 2010 and winter 2011. The effect of antibiotic treatment on bacterial community was investigated by comparing laboratory cultured *Porphyra* treated with penicillin and streptomycin, with wild *Porphyra* from the field. PCR products encompassing the V5 to V9 regions were partially sequenced and analyzed using the Ribosomal Database Project and BLAST. Sequences were assigned to seven taxonomic classes of the domain Bacteria: Sphingobacteria, Planctomycetacia, Gammaproteobacteria, Flavobacteria, Alphaproteobacteria and Actinobacteria. Preliminary results indicate that seasonal conditions impact the microbiota associated with the *Porphyra* blade, and that, not surprisingly, antibiotic treatment strongly biases the population. High-throughput sequencing is currently in progress to profile the complex microbial communities associated with these *Porphyra* blades with the goal to understand how the various members of the bacterial community impact the growth and development of *Porphyra umbilicalis*.

PHYTOPLANKTON BLOOMS ON THE CONTINENTAL SHELF OF WESTERN AUSTRALIA

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The continental shelf of Western Australia supports a winter phytoplankton bloom that is more intense during La Niña years. The coastal station at ~32°S has a ~60 year record showing warmer winter conditions and greater nitrate concentrations. On this downwelling coast the source of dissolved nitrogen to support the phytoplankton bloom was recently shown to be a thin, shallow layer of intense nitrification. The regions of high primary production were associated with this unusual source of nitrogen and various features of the Leeuwin Current. Several unusually productive oceanic stations had this shallow and thin layer of high nitrate below the mixed layer but within the euphotic zone. Oceanic stations were dominated by the picoplanktors, *Synechococcus* and *Prochlorococcus* but areas of greater primary production were more diverse; a community with more diatoms, chlorophytes, prasinophytes and cryptophytes. Large meanders formed warm-core eddies that also had a shift in the phytoplankton community towards diatoms. Photosynthetic dinoflagellates were negligible but heterotrophic and kleptochloroplastic taxa were common. Coccolithophorids and pelagophytes were also common but seemed to contribute little to the geographic variation in primary production.

CHANGES IN DISTRIBUTION AND ABUNDANCE OF THE KELP SPECIES *MACROCYSTIS* AND *NEREOCYSTIS* IN WASHINGTON STATE

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Two kelp species, *Nereocystis luetkeana* and *Macrocystis pyrifera* (Phaeophyceae, Laminariales) form extensive beds in the nearshore waters of Puget Sound, Washington. Because the pneumatocyst-bearing sporophyte phases form conspicuous surface canopies, they are easily mapped. We have mapped these species annually since 1989 along the Strait of Juan de Fuca and outer coast. Additionally, historical maps date from the late 1800's on the ''first t-sheets'' a comprehensive survey in 1912–15, and other subsequent surveys. These surveys show that while the fidelity of the location of ''beds'' has been remarkably consistent, inter-annual variation and long-term changes have been substantial. Abundance of both species has increased along the outer coast and Strait of Juan de Fuca since 1989. As elsewhere, high inter-annual variability appears to be linked to ENSO events. In the last century, there has been localized losses of *Nereocystis* in the San Juan Archipelago, and significant losses in central and southern Puget Sound. Changes may be due to grazing pressure, water quality changes, and community shifts following disturbances. Kelp losses have implications for the recovery of ESA-listed species of salmon and rockfish.

ACCLIMATED PHYSIOLOGY OF THE DIATOM *THALASSIOSIRA PSEUDONANA* UNDER PRE-INDUSTRIAL AND FUTURE LEVELS OF CO_2: IMPLICATIONS FOR CARBON SEQUESTRATION

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Diatoms are responsible for 20% of global primary productivity; some of this carbon sinks and is buried by sediments resulting in carbon sequestration. Studies have suggested that diatoms may fix more carbon under future levels of high CO_2, increasing the Redfield ratio (C:N) of sinking material and increasing the amount of carbon sequestered. To investigate this hypothesis we conducted chemostat experiments with *T. pseudonana* to acclimate the cells to pre-industrial levels of CO_2 (Media 600 ppm, Chemostat 300 ppm), and future levels of CO_2 (Media 1150 ppm, Chemostat 500 ppm). *T. pseudonana* was limited by 10 μM nitrate with dilution rate controlling growth rate to 1.5 per day. We collected particulate and dissolved organic carbon, carbonate chemistry and flow cytometry measurements. The Redfield ratio did not change significantly between CO_2 treatments, but the relative uptake of CO_2 increased under the high CO_2 treatment. Flow cytometry showed a decrease in cell volume at high CO_2. These results suggest a change in carbon uptake from bicarbonate to CO_2, however the Redfield ratio of diatoms acclimated to high CO_2 will not change and therefore carbon sequestration will not increase.

CHANGES IN PHOTOSYNTHESIS AND PRIMARY PRODUCTION OF PHYTOPLANKTON UNDER FUTURE CLIMATE CONDITIONS: A MESOCOSM STUDY

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A mesocosm experiment was conducted to evaluate the effects of future climate conditions on photosynthesis and production of phytoplankton community. Natural phytoplankton assemblages were incubated with nine mesocosms under the present conditions (ca. 400 ppmv CO_2 and ambient temperature), and two conditions of the future climates (acidification: ca. 900 ppmv CO_2 and ambient temperature; greenhouse: ca .900 ppmv CO_2 and ~3 °C warmer than ambient). Light utilization efficiency (α, LC) and maximum relative electron transport rate (rETRm, LC) significantly reduced at the acidification condition during the bloom of phytoplankton, but photosynthesis and primary production were not affected by the manipulated future conditions. The future climate conditions repressed the biomass growth via enhancing the grazing activity during the bloom period. If these results are in expressive future climate responses, the ability of light utilization could be declined at increased CO_2 conditions without down-regulation of photosynthesis. Also we propose that grazing pressure could be a key factor for regulation of phytoplankton bloom in future oceans.

EFFECT OF SALINITY ON GROWTH AND SURVIVAL OF EIGHT SPECIES OF *GAMBIERDISCUS*

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The genus *Gambierdiscus*, which includes >10 species of benthic/epiphytic dinoflagellates, has been recognized as the source of toxins causing ciguatera fish poisoning (CFP). The spatial and temporal unpredictability of CFP outbreaks has been ascribed to blooms of different *Gambierdiscus* species with different toxicities and habitat requirements. Recent progress in molecular identification of *Gambierdiscus* species has made it possible to determine the effects of environmental variables, such as salinity, on growth and distribution of *Gambierdiscus* species. However, the salinity vs. growth relationship for each species needs to be defined before this factor's effect on species distribution can be understood. Here we report the results of laboratory experiments testing the effect of salinity on growth of eight species of *Gambierdiscus*. Maximum growth rates of each species were measured across a range of salinities between 15 and 41 and growth rates were used to define optimum salinity (Smax) for each species as well as upper (SHi) and lower (SLo) tolerance limits for survival. We also used sudden decreases in salinity to simulate the effect of freshwater input on growth and survival.

GENETIC STRUCTURE OF THE TOXIC DINOFLAGELLATE *ALEXANDRIUM OSTENFELDII* IN THE BALTIC SEA

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The toxic dinoflagellate, (*Alexandrium ostenfeldii*), (Paulsen) Balech & Tangen has recently started to form dense localized blooms also in the central and northern parts of the Baltic Sea. To better understand the present expansion of the species in the Baltic Sea, we analyzed the genetic structure and gene flow among different spatially and temporally separated populations using 1177 polymorphic AFLP-markers. Fragment analyses revealed high genetic variability within all examined populations. Spatial populations were significantly differentiated from each other with the highest level of differentiation measured between the Southern and Northern populations. Such clear geographic structure suggests some degree of local adaptation despite the relatively low level of genetic difference and apparent gene flow among the populations. Cyst and bloom populations from the same location displayed nearly equal genetic structures. The high genetic diversity of the bloom population was unexpected since cyst populations are usually considered genetic reservoirs and more diverse than fast- growing bloom populations. We assume that sexuality in bloom populations might play a more important role than previously thought in maintaining the genetic diversity of the population.

FRESHWATER HABS IN WASHINGTON STATE

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Toxic blue-green algae (cyanobacteria) are an emerging public health concern in Washington's recreational waters causing illnesses to humans, pets, and wildlife. Washington Department of Health (DOH) developed provisional guidance values for microcystins (6 ug/L) and anatoxin-a (1 ug/L) for incorporation into a protocol for lake managers. DOH and Washington Department of Ecology work with local health partners to track toxic blooms and related animal and human health incidents throughout the state. Microcystins were observed above the guidance value in 18 lakes in 2008, 19 lakes in 2009, and 14 lakes in 2010 (maximum = 18,700 ug/L). Anatoxin-a was observed above the guidance value in eight lakes in 2008, 4 lakes in 2009, and 5 lakes in 2010 (maximum = 172,640 ug/L). Cylindrospermopsin (maximum = 0.106 ug/L) and saxitoxin (maximum = 193 ug/L) have each been observed in two Washington lakes. Partners are beginning a third season of monitoring thirty Puget Sound lowland lakes for the four cyanotoxins as part of a cooperative agreement with Centers for Disease Control and Prevention entitled ''Harmful Algae Bloom-related Illness and Surveillance System'' (HABISS).

SEASONALITY OF SELECTED NUTRITIONAL CONSTITUENTS OF EDIBLE DELMARVA SEAWEEDS

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Selected proximate constituents of three edible seaweed species (*Ulva lactuca*, *Fucus vesiculosus*, and *Gracilaria tikvahiae*) were compared for possible development as a food crop on the Delmarva Peninsula region. Sampling was conducted bimonthly in Virginia and Delaware from 2005–2008. Moisture, ash, dietary fiber, proteins and fat were measured seasonally and calorific value calculated once. Statistically significant variations in the selected proximate constituents were found among seasons, species, and between and within sites. The brown seaweeds (*Fucus*) at both sites had higher fiber, fat, and ash (mineral) content than green (*Ulva*) or red (*Gracilaria*). The red and green seaweeds had higher protein content than brown seaweeds. Positive correlations between seaweed fat and protein content indicate increases in the synthesis of both components under optimal growth conditions. Differences in species physiology and water quality at the two sites produced the differences in proximate constituents. This study contributed new information about the nutritional content of Atlantic coast seaweeds in the wild and may promote the use of seaweed as a regional crop.

A NEW GENUS OF ENDOSYMBIOTIC DINOZOANS FROM LARGER FORAMINIFERA

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Fine structural and molecular data suggest that the endosymbiotic dinoflagellates of soritine foraminifera belong to two separate, but related genera. The new genus is described in hospite from its host *Marginopora* spp. In addition to having petal-like plastids, it has a nucleomorph in its plastid, a nucleolus, and membranous bodies, structures not previous identified in members of the genus *Symbiodinium.* Although not yet characterized, enzymatic studies suggest chemical differences between the amphiesma of *Symbiodinium* and the new genus. Preliminary data from searches to find criteria that may be useful in assigning specific epithets to other soritid symbionts are discussed. Overall size was a potential character, as were life cycle behavior, settling pattern of zoospores, characteristics of the cell surface, chromosome numbers, nuclear to cell volume ratio, plastid architecture, presence or absence of membranous bodies, nucleoli, or a nucleomorph, and phylotypes.

UNRAVELING THE TAXONOMIC KNOT OF *BRACTEACOCCUS*, *DICTYOCOCCUS*, *PSEUDOMURIELLA*, AND *CHROMOCHLORIS* (CHLOROPHYCEAE, CHLOROPHYTA): A CASE OF CRYPTIC GENERA

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Bracteacoccus, a common soil alga, is taxonomically interwoven with the morphologically similar *Dictyococcus, Muriella* and *Pseudomuriella.* This has raised questions about the validity of these genera and the proper taxonomic disposition for their species. Phylogenetic relationships for a number of these taxa can be addressed using molecular sequence data obtained from type strains. Using analyses of 18S and rbcL, we determined that monophyly of *Bracteacoccus* is achieved only by excluding four unrelated lineages that historically have been included under this name. Firstly, *B. engadinensis* belongs in *Pseudomuriella,* along with *Dictyococcus schumacherensis.* Secondly, *B. cinnabarinus* and *B. minutus* group with *Muriella zofingiensis* in a lineage distinct from *Muriella* (Trebouxiophyceae). We assign them to the resurrected genus *Chromochloris* (Chlorophyceae). Thirdly, our results corroborate the transfer of *B. irregularis* into *Myrmecia.* Three other strains deposited in the UTEX collection as *Bracteacoccus* also belong to *Myrmecia.* Lastly, *Dictyococcus* is an unrelated lineage that can be reliably distinguished from *Bracteacoccus* based on chloroplast morphology. Our results indicate that *Pseudomuriella, Chromochloris* and *Bracteacoccus* are cryptic genera, representing closely related yet divergent lineages.

A MOLECULAR PHYLOGENY AND REEVALUATION OF THE POST-FERTILIZATION STAGES OF THE GENUS *CALLOPHYLLIS* (KALLYMENIACEAE, RHODOPHYTA)

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Previous studies indicated that *Callophyllis* has a procarpic type of the cystocarp development, in which the supporting cell functions as the auxiliary cell after fertilization and the gonimoblast initials are produced directly from the fusion cell. In this study, we have examined *Callophyllis variegata* and *C. pinnata* from Chile and '*Pugetia*' *delicatissima* from New Zealand and reevaluated their post-fertilization stages. In the *Callophyllis* species, the subsidiary cells enlarge and become highly lobed after fertilization. One of the lobed subsidiary cells cuts off two or more small connecting cells. Each connecting cell passes a diploid nucleus to a neighboring subcortical cell. Diploidized subcortical cells in turn cut off connecting cells that fuse with other gametophytic cells and pass on diploid nuclei. At the same time they produce gonimoblast initials. The cells of the supporting cell system eventually unite into a fusion cell and the gonimoblast initials form filaments that ramify through the medulla and bear carposporangia in clusters at maturity. We will briefly compare the post-fertilization stages in *Callophyllis* and *Pugetia* and discuss their molecular phylogeny.

PHYLOGENY OF THE PEDINOPHYCEAE (CHLOROPHYTA)

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The Pedinophyceae Moestrup is an assemblage of tiny green flagellates with a single long flagellum and a second flagellum represented by only a basal body. This class comprises the genera *Pedinomonas*, *Resultor*, and *Marsupiomonas* as the only known representatives. Ultrastructural features of these organisms led to the conclusion that the class is one of the ''primitive'' lineages of green algae. In this study, we examined the phylogeny of the Pedinophyceae using 18S rDNA and rbcL sequence data. Results of our analyses provide evidence that the Pedinophyceae are monophyletic and are related to the Chlorophyceae, Ulvophyceae and Trebouxiophyceae. However, results are inconclusive concerning the relationship of the Pedinophyceae to the other core chlorophyte classes. Our results indicate that these genera are neither ''primitive'' nor basal in the green algae, but rather are highly reduced and specialized forms. We propose a new family, Marsupiomonaceae, and new order, Marsupiomonales, to include those members of the class that possess a flagellar pit and theca.

SYSTEMATIC INVESTIGATION OF THE FRESHWATER RED ALGAL GENUS *THOREA* (THOREALES, RHODOPHYTA)

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In the past two decades, the genus *Thorea* has been revised several times and the number of recognized species has ranged from as few as four to as many as 14. However, there has been limited exploration of molecular species circumscription to date. The *rbcL* and LSU genes were sequenced for *Thorea* samples from Europe, North America, and the South Pacific. This data set was combined with published sequence data from South America, Asia, and Australia. All data were analyzed using Maximum Parsimony, Bayesian Inference, and Maximum Likelihood. Analysis of species examined to date shows two major evolutionary lineages within *Thorea*, each lineage with previously described species and potential new taxa. Additionally, specimens assigned to *T. violacea* are paraphyletic and represent both previously described, synonymized taxa and at least two additional taxa from the Dominican Republic and New York. Molecular data supports a greater amount of diversity then previously understood in the genus *Thorea*. Additional data, including the *cox*1 marker, and future research will be discussed.

PHYLOGENETIC AFFINITIES AND BIOGEOGRAPHY OF THE HAWAIIAN FRESHWATER RHODOPHYTA

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As the most isolated island archipelago in the world, the Hawaiian Islands provide a unique system for studying evolution and biogeography. Organisms arrived to the Hawaiian Islands through dispersal events from the large continental landmasses of the Americas and Austral-Asia. Since these dispersal events are putatively rare, many groups in the islands exhibit high levels of endemism. While much is known about the distributions and origins of land plant and animal systems, relatively little is known about the freshwater algae flora. Here, we present data on the phylogenetic affinities of the most common macroalgal freshwater rhodophytes in Hawaii, *Compsopogon caeruleus* and *Chantransia*, the sporophyte

stage of species in the Thoreales and Batrachospermales. We document four types of *Chantransia*: two previously reported from Hawaii and two new to this study. Molecular data reveal contrasting phylogeographic patterns in these rhodophytes within the Hawaiian Islands and through comparisons to previously published data, we are able to make inferences on the origins and distributions of these species beyond the central Pacific.

THE SURPRISINGLY DIVERSE WORLD OF ANAEROBIC HETEROLOBOSEA (EXCAVATA)

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Heterolobosea is ecologically extremely diverse group of protists. Some heteroloboseans flourish in hypersaline or extremely acidic habitats, while others are obligate or facultative anaerobes. To study the diversity of anaerobic Heterolobosea, we have cultivated 55 strains of free-living heteroloboseans from sediments around the world. The phylogenetic analysis of the SSU rRNA gene showed that all but one strains belong to the main anaerobic clade of Heterolobosea (Psalteriomonadidae). We identified three new genera and four new species. We showed that *Percolomonas descissus* is not related to *P. cosmopolitus*, but belongs to Psalteriomonadidae as well. The real diversity of Psalteriomonadidae is thus at least twice as wide as previously assumed. At least one lineage of *P. descissus* is restricted to the ''circumequatorial belt''. The remaining isolate of anaerobic Heterolobosea, PC4AM, is rather interesting. Its cell structure and life cycle is unique and it is impossible to classify this organism into any eukaryotic group solely on the basis of morphology. Concatenation of three genes showed that PC4AM is a heterolobosean, possibly closely related to Stephanopogon. It represents a novel anaerobic lineage of Heterolobosea.

NEW, RESURRECTED AND REDEFINED SPECIES OF *MASTOCARPUS* (PHYLLOPHORACEAE, RHODOPHYTA) FROM THE NORTHEAST PACIFIC

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Recent molecular phylogenetic investigations of the red algal genus *Mastocarpus* Kützing from the northeast Pacific resolved numerous cryptic species. Although species were clearly defined through genetic analyses, the correct names to apply to the species remained unclear due to morphological variability exhibited by algal thalli. To determine the appropriate name for each entity, we analyzed DNA from type material of taxa previously ascribed to *Mastocarpus*. Matching partial ITS sequences of type specimens to modern collections, we are able to apply the currently used names *Mastocarpus papillatus*, *M. pacificus* and *M. jardinii* to the appropriate species. We resurrect the following names and propose new combinations in *Mastocarpus* for *Gigartina cristata*, *G. latissima*, and *G. agardhii* and create new names for species for which we were unable to verify an existing name. The name *M. jardinii* applies to a species thus far collected only from Moss Beach, San Mateo Co. and the Monterey Peninsula, California. Specimens previously assigned to *M. jardinii* are now separated into three species: two new to science and one previously described. An updated phylogeny using a broad range of geographical and morphological collections is presented that includes data from three genomes: nuclear ribosomal ITS, chloroplast *rbcL* and mitochondrial *COI*. Morphological and anatomical diagnoses, along with vertical distributions and geographic ranges, are provided for each species.

ECOLOGICAL DIFFERENTIATION OF CRYPTIC SPECIES IN GREEN MICROALGAE

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The green microalgae are one of the most diverse groups of protists. However, our understanding of

their real diversity is still very incomplete. We used two cosmopolitan, widely distributed genera of microscopic green algae – *Asterochloris* (Chlorophyta) and *Klebsormidium* (Streptophyta) – to study the cryptic diversity in morphologically uniform taxa. Phylogenetic analyses of a large number of isolated strains revealed extensive genetic diversity in both studied genera. Even that the strains were morphologically highly similar, some phenotypic characters appear to be suitable for delimitation of particular lineages. Further, the cryptic lineages exhibited strong ecological preferences to specified environmental factors. In *Klebsormidium*, the strains were clustered according to the preferred habitat types. In *Asterochloris*, particular lineages were distinguished according to their substrate and climatic preferences. Our data thus clearly indicate that the morphologically defined species of green algae could in fact consist of a large number of cryptic species, exhibiting clear ecological preferences for various environmental factors. The lack of suitable morphological characters then masks the real species diversity, which is manifested by the substantial genetic diversity in molecular markers.

ULTRASTRUCTURE, ZOOSPOROGENESIS, PIGMENT COMPOSITION AND PHYLOGENETIC POSITION OF *TRACHYDISCUS MINUTUS* (EUSTIGMATOPHYCEAE, HETEROKONTOPHYTA)

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The traditional order Mischococcales (Xanthophyceae) is polyphyletic with some original members now classified in a separate class Eustigmatophyceae. It is probable that many additional mischococcalean species may prove to be misplaced upon closer investigation. We established an algal culture determined as one such species, *Trachydiscus minutus* (Bourr.) H.Ettl, and studied it in detail. We discovered a zoosporic part of the life cycle of this alga, which was induced by darkness, suppressed by light and was temperature dependent. The zoospores possessed one flagellum with mastigonemes and a putative basal swelling, but a stigma was missing. Plastids in vegetative cells lacked both a connection to the nuclear envelope and a girdle lamella. Pigments of *T. minutus* included violaxanthin and vaucheriaxanthin (ester) as the major carotenoids; we detected no chlorophyll c. An 18S rRNA gene-based phylogenetic analysis placed *T. minutus* in a clade with species of the genus *Pseudostaurastrum* and with *Goniochloris sculpta*, which formed a sister branch to initially studied Eustigmatophyceae. Our results show that *T. minutus* is not a xanthophycean and place it into a novel deep lineage of the Eustigmatophyceae.

WHAT IS *OOPHILA* AND ARE GREEN ALGAE ASSOCIATED WITH SALAMANDER EGGS MONOPHYLETIC?

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Associations of diverse green algae with ciliates, invertebrates, and several salamander species have been known for over 100 years. *Oophila* was described in 1909 for algae that colonize salamander eggs. This appears to be a mutualism where the algae play a role in salamander development by increasing the oxygen available to embryos while the algae use the embryos as a nitrogen source. Recently, an even more intimate association of green algae and salamanders was described, one in which the algae occur inside tissues and even cells of the developing salamander. Our phylogenetic analyses using 18S rDNA data from green algae isolated from salamander eggs growing in New York, Connecticut, Kentucky, and Louisiana indicate that all of the sampled algae are members of Chlorophyceae. However, this algal assemblage does not form a monophyletic group but is instead phylogenetically and phenotypically diverse. In light of the evidence that vertical transmission of algae from parent salamanders to embryos may be occurring, our results raise intriguing questions regarding the specificity and evolution of the algal-salamander symbiosis.

IDENTIFYING LATIN AMERICAN *CHARA* (CHARALES: CHAROPHYTA) USING DNA SEQUENCES

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The New York Botanical Garden Herbarium (NY) houses more than 500 specimens of *Chara* collected from Latin America. These specimens were catalogued and their collection information and taxonomic annotations were made available online (sciweb.nybg.org/science2/hcol/chara/index.asp). DNA was extracted from 339 specimens and attempts were made to amplify and sequence the plastid genes *atpB* and *rbcL*. Resulting sequences were incorporated into a larger data set that included numerous *Chara* species from around the world. Thirty-four percent (116) of the specimens were successfully sequenced to some extent. Sixty-six percent (76) of those specimens were analyzed phylogentically. Of those specimens, the molecular data were consistent with the original determination for ~49% (37), and inconsistent for ~43% (33). Six previously unidentified specimens were identified to species with these data. Due to incomplete sequence data, we were unable to identify ~36% (42) of the specimens to species using phylogenetic methods. However, we were able to place these individuals into a broader phylogenetic context (e.g. sections). These data contribute to our understanding of the occurrence and biogeographic patterns of *Chara* in Latin America.

GENETIC DIVERSITY OF CILIATE MORPHOSPECIES

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The relationship between morphospecies and genetic diversity remains unexplored for most eukaryotic microbes, including many ciliates. Ciliates are microbial eukaryotes that are characterized by the presence of cilia in at least one of their life stages and by their nuclear dimorphism (i.e. they have a somatic macronucleus and a germline micronucleus). Although our knowledge about ciliate diversity has increased with the application of molecular tools, further analyses are needed to understand their phylogeny and phylogeography. For this study, we are using multiple markers to investigate the genetic diversity underlying ciliate morphospecies, using as a model the species *Chilodonella uncinata*. Because of their amplified macronuclear genome, we can reliably characterize multiple genes from single cells. Hence, we are sequencing both the nuclear and mitochondrial SSU-rDNA genes from cells isolated across time and space. Preliminary results reveal many genetic forms with complex phylogeography within morphospecies.

NEW TAXONOMIC CONCEPTS IN THE GENERA OF THE PEYSSONNELIACEAE (PEYSSONNELIALES, RHODOPHYTA) WITH FOCUS ON TAXA FROM THE GULF OF MEXICO AND PANAMA

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Post-oil spill impacts of the anthropogenic April 2010 BP Deepwater Horizon crude oil spill resulted in a dramatic decrease of seaweed diversity offshore Louisiana, Mississippi and Alabama in the northwestern Gulf of Mexico. The few taxa that were dredged in these areas in December 2010 and April 2011 were crust-forming members of the Peyssonneliaceae, i.e. *Peyssonnelia inamoena* and *Riquetophycus* spp. The generic concepts of these taxa will be critically assessed in a worldwide phylogeny of the family on the basis of comparative chloroplast-encoded rbcL and nuclear LSU rDNA sequence analysis. Diagnostic vegetative and reproductive morphological characters of other new taxa of Peyssonneliaceae from the Gulf of Mexico and Panama will be highlighted as well.

MIXOTROPHIC PROTISTS IN HIGH ARCTIC LAKES

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Light limitation is an important constraint on photosynthetic protists in Arctic lakes for much of the year, due to ice cover and polar darkness. Limited primary production should lead to a simple aquatic microbial food chain. However, mixotrophic chrysophytes dominate these ecosystems, hinting at a more complex food web. Either the strong seasonal light variability or the low nutrient levels could favor the persistence of mixotrophs. A dilution experiment was conducted comparing communities placed in the dark compared with those left at ambient summer light levels. Communities were followed via both pyrosequencing of 18S rRNA and microscopy. The response of both small photosynthetic plankton and bacteria to the dilutions was consistent with grazing pressure on the two functional groups. Growth rates were greater for heterotrophic protists in dark-bottles while mixotrophs increased in the light bottles. We concluded that mixotrophic protists were more competitive grazers under favorable light conditions. The absence of light, however, favored heterotrophs. We suggest that the ubiquity of mixotrophs in these arctic lakes in summer may be better explained by their use of grazing to supplement photosynthesis limited by nutrient supply.

DIVERSITY AND ECOLOGY OF APUSOZOA (PROTOZOA), A MYSTERIOUS PHYLUM OF FREE-LIVING ZOOFLAGELLATES

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Apusozoa is a recently established protozoan phylum primarily comprising biciliate gliding zooflagellates classified within three orders: Apusomonadida, Planomonadida, and Mantamonadida. Using sequences of known strains, we designed PCR primers specific to each order and obtained clone libraries from a range of natural environments. We found evidence of many new apusomonad and planomonad lineages, as well as novel mantamonad ITS1 ribotypes, from marine, freshwater, and soil environments. We show that Apusozoa is more diverse and cosmopolitan than revealed by culturing alone and that some lineages may be more ecologically sensitive than others. Our results suggest that *Mantamonas plastica*, the only described mantamonad species, thought to be exclusively marine, also exists in freshwater and soil. We also mined the 454 sequencing dataset produced by the European consortium BioMarKs for apuosozoan V4 sequence tags from European coastal waters. These results reveal much higher apusozoan diversity than our apusozoan-specific clone libraries, and allow us to compare lineages recovered from DNA vs cDNA libraries, planktonic vs. benthos, oxic vs anoxic, and different physio-chemical conditions.

EUGLYPHID DIVERSITY: BUSINESS AS USUAL OR TERRA INCOGNITA?

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Euglyphids are a group of cercozoan testate amoebae that secrete an ornamented shell whose shape is diagnostic for species identification. Together with Arcellinid testate amoebae, euglyphids are commonly used as bioindicators for environmental conditions (e.g. soil moisture, pH, P) and pollution (e.g. heavy metals) in present day- and palaeo-ecology. However, the proper use of organisms as bioindicators relies both on a sound taxonomy and on a clear idea of their geographical distribution. Our results prove that neither of these two conditions is met, and that the accepted estimation of 800 euglyphid species is way below the reality. Here, I will present a newly emerging picture of the diversity and biogeography of euglyphid testate amoebae based on SEM microscopy and molecular data.

SOME OBSERVATIONS ON THE PHTYOPLANKTON FROM AN OLIGOHALINE, LAGOONAL, SUBTROPICAL ESTUARY

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The Lake Pontchartrain basin estuary is a shallow, subtropical system located on the northern border of New Orleans, Louisiana. Rivers, bayous, swamps, and marshes feed two lentic water-bodies connected by an 11-km pass that drain to the Gulf of Mexico via a canal and two natural passes. This presentation summarizes several studies emphasizing the estuary's low salinity and lagoonal geomorphology. The silica-scaled chrysophyte flora is particularly diverse in the freshwater environments feeding and surrounding the estuary. Algal viruses encoding the *psbA* gene appear to be common. In the fall, in central Lake Pontchartrain, diatoms contributed about three times more to total chl *a* biomass than all other algal groups combined, while all algal groups contributed about equally in winter samples across the estuary. Diatom-dominated phytoplankton blooms occurred in central Lake Pontchartrain around the pumping-station outfall during the dewatering of New Orleans following hurricane Katrina, but chl *a* biomass and diversity indicators declined to moderate levels in about 40 days. One enigmatic diatom, *Chaetoceros subtilis* var. *abnormis*, occurs frequently in the estuary and may be characteristic of subtropical, oligohaline environments.

THERE'S MORE THAN CHLOROPHYLL IN A SUBSURFACE CHLOROPHYLL MAXIMUM LAYER

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A subsurface chlorophyll maximum layer is common in many pelagic regions and is ubiquitous in the salinity stratified Arctic Ocean. In the Beaufort Sea (Western Canada), the maximum peak tends to occur below the nutrient poor upper mixed layer within Bering Sea Pacific Winter Water, which is high in nitrate and silicate. Most studies have focused on pigment signatures of chlorophyll containing organisms in this layer and little is known of the taxonomic composition of the community. Molecular gene surveys have highlighted the importance of heterotrophic protists throughout the water column, but have tended to focus on surface or deep samples. Here we explored the diversity of protists in Arctic chlorophyll maxima using tag pyrosequencing to determine community structure in the upper, mid and lower reaches of the layer. The non-photosynthetic protists represent multiple trophic levels and suggest diverse complex microbial food webs within chlorophyll maxima, with implications for carbon fluxes, nutrient cycling and food availability for higher trophic levels.

THE ''PLASTISPHERE'': A NEW AND EXPANDING HABITAT FOR MARINE PROTISTS

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Plastic marine debris is a growing problem in the ocean. The 'Phycosphere' consists of microbes attached to algae and has been studied for many years, but plastic is an anthropogenic substrate that has only recently become available to colonizing protists. We documented the protist community on open ocean plastic debris samples using 454 tag pyrosequencing and scanning electron microscopy. Plastic samples showed considerable species richness and included representatives from many protist groups including fungi. Sequences recovered were dominated by heterotrophs including an unidentified sequence related to colonial radiolaria and suctorian ciliates, but many photosynthetic groups including dinoflagellates, diatoms, and green, brown, and red algae were also present. SEM images revealed a complex landscape of microbes on the surface of the plastic; diatoms and stalked suctorians covered with what appeared to be bacteria were among the sequenced protists recognized. We have proposed the term ''Plastisphere'' to describe the community of organisms attached to plastic debris in aquatic systems.

ANALYSIS OF A NUISANCE GREEN ALGAL BLOOM ON THE ORLAND RIVER, MAINE (USA)

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Green algae grew to nuisance levels in summer 2010 over 6 km of the Orland River estuary (10–22 PSU) at the head of Penobscot Bay (Maine). Fresh algal samples were dried, and DNA extracted with a Qiagen DNeasy Plant Mini Kit. Sequences for the universal plastid amplicon (UPA) and *rbcL* gene were obtained following PCR of diluted DNA by standard techniques. BLAST analysis indicated that the sequence recovered for the UPA was a diatom similar to *Nitzschia* spp. (EF426584.1; E = 0.0), whereas the *rbcL* sequence was an *Ulva* spp., as expected from tubular fragments in the sample. The *Ulva* spp. with highest similarity (EF110277.1, 99% similarity, E = 0.0) to the Orland sample was from New Zealand

(a possible *U. ralfsii* [type locality: Wales]), suggesting a potential non-native history. Studies of nutrient levels and water circulation patterns between the Orland and Penobscot Rivers are required to determine contributors to the bloom.

BIOGEOGRAPHY OF PROTOSTELOID AMOEBAE: AN ECOLOGICAL POINT OF VIEW

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Habitat availability and environmental preferences of species are the major factors determining the success of dispersal processes and the distribution of protists. We explored the differences in fundamental niches and potential distributions of an ecological guild of slime molds – protosteloid amoebae – in the Iberian Peninsula. A large set of samples collected in a northeast-southwest transect along the peninsula was used to test the hypothesis that, together with the existence of suitable microhabitats, climate conditions may determine the probability of survival of a species at a medium-large scale. Although protosteloid amoeba share similar morphologies and lifestyles, canonical correspondence analyses showed that they have varied niche preferences. Species colonizing various microhabitats can have different climatic optima in each of them, increasing their ability to tolerate larger climatic and geographic ranges, and suggesting a buffering effect on the influence of external climatic factors. Maxent environmental niche models provided predictions of the probability of presence of the species in areas that have not been sampled yet, and they were used to generate maps of potential distribution that were also compared.

THE ROLE OF THIN LAYER FORMATION IN THE SEXUAL REPRODUCTION OF THE PLANKTONIC DIATOM *CHAETOCEROS CONCAVICORNIS* IN MONTEREY BAY, CALIFORNIA

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Sexual reproduction is an inherent part of the life cycle of many diatoms. However, it is rarely observed *in situ*, and has not been documented for numerous taxa. Centric planktonic diatoms are primarily oogamous, whereby a motile male gamete must find a female gamete in a dilute environment. When spread homogeneously throughout the water column, these cells may be widely dispersed, with gametes failing to find each other. However, if the population is concentrated into a specific region of the water column, the chances of successful fertilization are likely to increase. Thin layers are highly concentrated patches of plankton a few centimeters to meters thick, which may stretch horizontally for many kilometers and persist in both space and time. During an interdisciplinary oceanographic project in Monterey Bay, California, we observed a thin layer dominated by *Chaetoceros concavicornis.* Nearly the entire population of this diatom was located within an approximately 2 m thick region of the water column. Male gamete formation, auxospores, and post-auxospore colonies were observed. The formation of thin layers may be critical to successful sexual reproduction in these organisms.

CULTIVATED AND CULTIVATION-INDEPENDENT DIVERSITY OF FUNGUS-LIKE MARINE PROTISTS, THE LABYRINTHULOMYCETES

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Labyrinthulomycetes are ubiquitous, abundant and diverse fungus-like osmoheterotrophic protists (heterokonts) that likely play an important role in the remineralization of particulate organic matter in the oceans. They also produce large amounts of essential polyunsaturated fatty acids (up to 25% of total cell biomass) *de novo.* We are using both molecular genetic methods and artificial media to investigate the diversity and abundance of labyrinthulomycetes in estuarine waters and sediments around Long Island, NY. Nearly full-length 18S rDNA sequences amplified with the labyrinthulomycete-specific primer LABY-Y revealed the presence of at least 10 novel types of labyrinthulomycetes, in addition to sequences closely related to previously cultivated aplanochytrids and oblongichytrids. Surprisingly few thraustochytrid sequences were recovered, which could reflect a bias of LABY-Y. However, a suite of complementary primers has revealed that the lack of thraustochytrid sequences accurately reflected a low abundance of these organisms. Our cultivation-dependent studies have shown

that different types of labyrinthulomycetes have strong preferences for different growth substrates (e.g. algal- vs crustacean-based media), which may underlie differences in the ecology of these organisms.

THE EFFECTS OF ENVIRONMENTAL AND CHEMICAL STRESSORS ON OXIDANT RELEASE BY TWO ULVOID ALGAE SPECIES IN WASHINGTON STATE

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Current global conditions are leading to increased numbers of harmful macroalgal blooms, which have detrimental effects on the ecosystems in which they occur. Macroalgae produce harmful chemicals such as oxidants, DMSP, and dopamine. These chemicals may be produced in high quantities in response to environmental stress. This project investigated the impacts of environmental (emersion, water temperature, hypoxia) and chemical (dopamine) stressors on oxidant production in two bloom forming macroalgae (*Ulvaria obscura* and *Ulva lactuca*). Oxidant concentrations decreased in *U. obscura* after emersion stress. Emersed algae produced oxidants at a rate that was one order of magnitude less than unemersed algae. Increased water temperature and dopamine concentration decreased oxidant production in *U. lactuca.* Algae exposed to 10° seawater produced more oxidants than algae in 15 and 20° seawater. Decreased oxidant concentrations of *U. lactuca* may have been caused by an increase in oxidant scavenging mechanisms while the decrease in *U. obscura* was possibly due to a decrease in cellular activity. As these two algae species occupy different tidal regions, their ability to handle stress should differ as well.

GROWTH RATES FOR THE KELPS *AGARUM CLATHRATUM*, *SACCHARINA GROENLANDICA*, AND *EUALARIA FISTULOSA* IN KACHEMAK BAY, ALASKA – DIFFERENCES IN LIFE HISTORY STRATEGIES

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Growth rates of the kelps *Agarum clathratum*, *Saccharina groenlandica*, and *Eualaria fistulosa* in Kachemak Bay, Cook Inlet, Alaska, were measured for 18 month in 1977–78. They changed dramatically seasonally. They were higher from late winter through mid-summer, peaking in May/June, but were lower from late summer through mid-winter.

Eualaria fistulosa, the largest but shortest-lived of the three species, grew fastest. *Agarum clathratum*, the smallest, grew slowest. Both *A. clathratum* (maximum total length [MTL] $\asymp$1.2 m) and *S. groenlandica* (MTL $\asymp$2.6 m) are perennials; *E. fistulosa* (MTL >25 m) is effectively an annual. Thus, growth rates appear to correlate positively with relative size, but inversely with longevity. Temporal growth patterns corresponded closely with those reported for species of Laminariales in the Arctic, Atlantic and Mediterranean. Patterns in stipe growth varied by species and season. For *A. clathratum,* they suggest that most occurs when plants are young; stipes of most measured plants grew little. In contrast, stipe length of many *S. groenlandica* and *E. fistulosa* plants doubled or tripled during the spring/summer growth period, but became shorter during the winter.

AEROTERRESTRIAL *COLEOCHAETE* MODELS EARLY PLANT ADAPTATION TO LAND

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The streptophyte water-to-land transition was a pivotal event in Earth history though poorly understood. While some early-diverging modern streptophyte algae are aeroterrestrial (capable of living in subaerial habitats), the complex streptophyte alga *Coleochaete*, identified by some as sister to the embryophytes, is generally regarded as obligately aquatic. We tested the ability of two *Coleochaete* species to grow and reproduce asexually in non-aquatic subaerial conditions and resist chemical degradation–a trait indicative of fossilization potential. When grown on agarized mineral media or on quartz sand, *Coleochaete* occurred as distinctive hairless multistratose hemispherical bodies having unistratose lobes or irregular clusters of cells with thick, layered chemically resistant walls. When exposed to liquid water, aeroterrestrial *Coleochaete* produced typical biflagellate zoospores. Cells that had been air-dried for periods up to several months maintained integrity and retained green pigmentation. The ultrastructure of acetolyzed aeroterrestrial *Coleochaete* resembled that of certain enigmatic Cambrian spore-like structures. Structural and

reproductive features of modern aeroterrestrial *Coleochaete* suggest that ancient complex streptophyte algae could have grown and reproduced in moist terrestrial habitats, persisted through periods of desiccation, and left distinctive fossilizable remains.

THE BLACK QUEEN HYPOTHESIS AND THE EVOLUTION OF ALGAL/BACTERIAL MUTUALISMS

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Mutualistic interactions between algae and heterotrophic bacteria often occur, despite theoretical predictions that such relationships should be unstable. We present a testable theory of co-evolution, the Black Queen Hypothesis (BQH), which can produce mutualisms between unrelated organisms through classical natural selection. Some essential biological functions are difficult to perform selfishly; i.e., some of their products are available to other nearby organisms. Organisms may increase their fitness by not performing such a function so long as some members of the community continue. BQH predicts that members of a community will evolve function loss until the benefits of loss are balanced by the costs, resulting in communities comprised of fast-evolving ‘‘beneficiaries’’ dependent on less mutable ‘‘helpers’’. When the beneficiary is an autotroph, such as an alga, and the helper is a heterotroph dependent on algal photosynthesis, the evolved relationship may appear mutualistic: decreased resource demands for the alga improve primary production, potentially increasing the carrying capacity of the community as a whole. Importantly however, BQH evolution is driven by classical natural selection on individual organisms; interspecies interactions emerge purely as by-products.

SELECTIVE FEEDING AND FOREIGN PLASTID RETENTION IN AN ANTARCTIC DINOFLAGELLATE

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A dinoflagellate strain (RSD) isolated from the Ross Sea, Antarctica was previously shown to feed on and sequester plastids from the haptophyte alga *Phaeocystis antarctica.* We followed populations of RSD in long-term co-culture with *P. antarctica* in separate batch culture experiments with either a polar haptophyte *Chrysochromulina* spp., or with the cryptomonad *Geminigera cryophila* (the plastid source for an Antarctic *Mesodinium rubrum*). Over a period of 16 days, RSD selectively fed on *P. antarctica,* substantially reducing its abundance, but no ingestion of *Chrysochromulina* spp. or *G. cryophila* by RSD was detected. Additionally, a population of RSD cells isolated from *P. antarctica* using flow cytometry grew slowly during 9 months with a marked reduction in mean numbers of chloroplasts/cell and an increased frequency of cells lacking chloroplasts. Together, these results suggest a feeding specificity and a longevity of the association that are consistent with the proposal that RSD represents a model for an early stage of plastid evolution.

DISTRIBUTION OF MACROALGAE AND SEDIMENT CHLOROPHYLL A ALONG SALINITY AND ELEVATION GRADIENTS IN OREGON TIDAL MARSHES

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Algae contribute to trophic and biogeochemical processes in tidal wetlands. We investigated patterns of sediment pigment content and macroalgal abundance and diversity in marshes in four Oregon estuaries representing a variety of vegetation types, salinity regimes, and tidal elevation. Common macroalgae included *Ulva, Rhizoclonium, Vaucheria, Fucus,* and *Porphya.* Macroalgal abundance and diversity was strongly linked to tidal elevation, with lower marshes containing more macroalgal species and greater algal cover. Vascular plant diversity, in contrast, increased with tidal elevation. Soil salinity measurements were coupled with elevation data to determine envelopes of environmental tolerance for common macroalgal taxa. As with macroalgal abundance, sediment chlorophyll a concentrations were much higher in lower tidal marshes. Qualitative observation of sediments suggested a diversity of diatoms and cyanobacteria were present which contributed to chlorophyll a content. Estimates of pheopigment concentrations usually exceeded chlorophyll a concentrations, probably because of substantial plant detritus in sediments. Understanding the spatial distribution of micro- and macroalgae in marshes will aid in quantification of ecosystem processes and prediction of marsh function with future sea level rise.

STABILITY AND EFFICIENCY OF MIXED ALGAL CULTURES

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The literature suggests that stable mixtures mixed cultures of algae are possible under certain circumstances. We seek to achieve this for the purpose of biofuels production, one approach suggested in the National Algal Biofuels Technology Roadmap. We will discuss the rationale, experimental design, and preliminary results for bench-scale mixtures of (initially) two species from different classes, and the prospects for eventual scale-up and process control to maintain long-term stability of productivity and species balance under variable natural conditions. Our approach is to directly monitor and control species balance, photosynthetic efficiency, and feedstock content (e.g. lipids) by manipulating selected conditions with minimal energy input.

CONSTITUTIVE DEFENSE IN THE MICROBIAL PLANKTON: A GIANT CELL SURFACE PROTEIN IN *SYNECHOCOCCUS* WH8102 INHIBITS FEEDING BY A MODEL DINOFLAGELLATE PREDATOR

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We used mutants of Sargasso Sea *Synechococcus* strain WH8102 (clade III), a picocyanobacterium, to test the hypothesis that cell surface proteins play a role in defense against predation by protists. Predation rates by the heterotrophic dinoflagellate *Oxyrrhis marina* on mutants lacking the giant SwmB protein were always higher (by 1.6 to 3.9x) than those on wild-type WH8102 cells, and equaled predation rates on a clade I strain (CC9311). In contrast, absence of the S-layer SwmA protein had no effect on predation by *O. marina*. Similarly, *Synechococcus* culture filtrates, known to contain dissolved SwmA and SwmB proteins, did not alter predation on WH8102, indicating that the deterrence mechanism involves cell-associated rather than dissolved substances. Measured differences in hydrophobicity did not account for SwmB's protective effect. We hypothesize that the giant SwmB protein, constitutively expressed in *Synechococcus* WH8102, defends by interfering with attachment of dinoflagellate prey capture organelles. Strategies for resisting predation may contribute to the competitive success of different *Synechococcus* groups, and to the diversity of natural picophytoplankton assemblages.

THE EFFECT OF OCEAN ACIDITY ON THE ATTACHMENT OF CORALLINE ALGAL SPORES

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With rising levels of CO_2, the oceans are expected to drop 0.3–0.4 units of pH over the next 100 years. Additionally, pH can vary daily by up to 0.6 units locally in Monterey Bay due to upwelling and other ocean processes. Several community level studies have found abundance of coralline algae to be reduced compared to non-calcifying algae in acidic conditions. In this study, we test the hypothesis that coralline spore attachment is inhibited by low pH, a possible cause for the reduced abundance of corallines in acidic conditions. Very little is known about the physics of algal spore attachment, so we will present some observations on the attachment process of *Corallina vancouveriensis*, a common intertidal coralline algae. Next we will test spores across a range of pH and settlement times using a newly designed shear flume. In this way, we will determine the effect that acidity has on both the maximum spore adhesion strength and the time it takes to reach this maximum strength.

MICROALGAL DIVERSITY FOR NATURAL WASTEWATER TREATMENT

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Present work highlights the scope and application of microalgal diversity of wastewater stabilization ponds with its potential for treating wastewater in terms of removal of organic and inorganic pollutants and heavy metal remediation. *Chlorella* sp. was present as one of the most dominating species in wastewater stabilization pond during whole 10 months on-site study at village Sanghol, Distt. Fatehgarh Sahib, Punjab, India. The order of microalgal dominance was *Chlorella* spp. > *Chlamydomonas* > *Lyngbya* > Diatoms, whereas *Chlorococcum* spp. and *Closteriopsis* spp.

during were also seen in the month of August and September besides cyanobacteria like *Gloeocapsa* and *Myxosarcina*. A marked reduction of 15 to 83% in BOD5 and 52 to 93% in COD from inlet wastewater after treatment represented effective treatment potential of wastewater stabilization ponds during complete sampling period. Metal removal studies carried out with pure culture of *Chlorella* sp. (R1) developed from pond wastewater demonstrated maximum removal potential for $Zn^{2+} > Pb^{2+} >$ Cr (total). Metal uptake capacity (qmax) of 34.36, 41.75, and 60.7 mg/g for Pb^{2+}, Zn^{2+} and total Cr for *Chlorella* spp. (R1) demonstrated good metal uptake potential. *Chlorella* sp. (R1) was analyzed for its effective role in removal of Cr from electroplating industrial wastewater using immobilized alga in packed bed column showed its strong potential for development of commercial bioresin.

EVOLUTIONARY REVERSALS: ARTICULATED CORALLINES *CHIHARAEA* AND *YAMADAIA* (CORALLINALES, RHODOPHYTA) REPRESENT A LOSS OF ARTICULATIONS AND REDUCTION OF STATURE

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The evolution of calcifying coralline algae has long interested biologists and geologists. Fossil and molecular analyses suggest that representatives of modern day crustose coralline algae (Corallinales and Sporolithales, Rhodophyta) evolved more than 100 million years ago and that these crustose corallines ultimately gave rise to upright, articulated coralline algae. Early studies by Johansen and Cabioch hypothesized that the articulated genera *Chiharaea* and *Yamadaia*, which produce extensive crusts and few diminutive fronds, may represent intermediates along the evolutionary continuum from crustose to articulated corallines. We test this 'intermediate frond hypothesis' for the first time by comparing *rbcL* gene sequences from generitype species *Chiharaea bodegensis* and *Yamadaia melobesioides* to sequences from other articulated and crustose corallines. We show that *Yamadaia melobesioides* belongs in a clade with Corallina and is unlikely to be a distinct genus. We show that *Arthrocardia silvae* and *Yamadaia americana* belong in a clade with *Chiharaea bodegensis*, likely representing three distinct *Chiharaea* species. We reject the 'intermediate frond hypothesis', and conclude that *Chiharaea* and *Yamadaia* evolved from articulated ancestors and exhibit a reduced stature due to loss of articulations.

GRATELOUPIA TAIWANENSIS IN ALABAMA: A NON-NATIVE SEAWEED IN THE GULF OF MEXICO

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Recent collections of seaweeds from the north central Gulf of Mexico have resulted in abundant individuals of red seaweeds found to be morphologically similar to *Grateloupia*. The specific identity of this alga has been investigated using a molecular approach using the chloroplast-encoded large subunit RuBisCO gene. Based on *rbcL* sequences and molecular phylogenetic analyses, these Alabamian specimens corresponded to *Grateloupia taiwanensis*, a species recently characterized from Taiwan.

MIXOTROPHY AND MORPHOSTASIS IN EUGLENIDS: ULTRASTRUCTURE AND MOLECULAR PHYLOGENY OF A NOVEL MARINE ISOLATE THAT IS BOTH PHOTOSYNTHETIC AND EUKARYOVOROUS

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Photosynthetic euglenids form a monophyletic group that evolved from phagotrophic ancestors via secondary endosymbiosis with green algal prey. Phagotrophic euglenids are equipped with a robust feeding apparatus consisting of rods and vanes. We established a culture of a novel marine euglenid using *Tetraselmis* spp. as a food source. This euglenid had an eyespot, chloroplasts, euglenoid movement, two flagella of unequal length, and the ability to feed by phagocytosis and myzocytosis. TEM demonstrated a reduced feeding apparatus consisting of a distinct rod of microtubules. When starved, most cells retained at least one chloroplast; however, cells could not live without *Tetraselmis* for more than 1 month. TEM of euglenoid cells demonstrated that two types of chloroplasts exist: one was from the consumed *Tetraselmis* cells and one contained internal pyrenoids penetrated by thylakoid membranes. Molecular phylogenetic analyses demonstrated that this euglenid branches as the

nearest sister lineage to the clade consisting of all photosynthetic euglenids. This phylogenetic context combined with the ultrastructural features conforms to the expected transitional stage that occurred during the early evolution of photosynthetic euglenids from phagotrophic (eukaryovorous) ancestors.

PHYLOGENOMICS OF HACROBIANS, WITH FOCUS ON CRYPTOPHYTES AND KATABLEPHARIDS

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Resolving the global tree of eukaryotes has been one of the most important challenges of evolutionary biology. Over the past few years, phylogenetic analyses of very large datasets (phylogenomics) have led to the proposals of unexpected relationships between the supergroups of eukaryotes. Some of these new relationships, such as the grouping of stramenopiles, alveolates and rhizarians appear to be very robust (the SAR group). Others, on the other hand, are highly debated hypotheses that are in urgent need for new supporting evidence. Here, I will discuss the strengths and weaknesses of one of these new controversial large groupings, Hacrobia. This putative assemblage has been recently proposed to comprise the secondarily photosynthetic haptophytes and cryptophytes, but also the non-photosynthetic lineages telonemids, centrohelids and katablepharids. In order to tackle this question, we constructed a new species-rich phylogenomic dataset of over 250 nucleus-encoded proteins which, most importantly, includes the information from the first completely sequenced cryptophytes nuclear genome (*Guillardia theta*) and a large EST dataset for the katablepharid species *Roombia truncata.*

VOLVOX (SECT. *MERRILLOSPHAERA*) *OVALIS* SP. NOV. (VOLVOCACEAE, CHLOROPHYCEAE) FROM TEXAS

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Smith (1944, Trans. Am. Microsc. Soc.) divided the familiar genus *Volvox* into four sections, placing seven species that lacked cytoplasmic bridges between adult cells in the section *Merrillosphaera.* Here we describe a new member of this section from Texas: *V. ovalis* M.A. Pocock ex H. Nozaki et A.W. Coleman sp. nov. Asexual spheroids of *V. ovalis* are ovoid or elliptical, with a monolayer of 1000–2000 somatic cells that are not linked by cytoplasmic bridges, and 8 to 12 gonidia in the posterior region. Visibly asymmetric cleavage divisions do not occur in *V. ovalis* embryos as they do *V. carteri*, *V. obversus* and *V. africanus*, so the gonidia of the next generation are not yet recognizable in *V. ovalis* embryos prior to inversion. Molecular phylogenetic analyses of the five chloroplast genes and the internal transcribed spacer regions of nuclear ribosomal DNA indicated that *V. ovalis* is closely related to *V. spermatosphaera* and/or *V. tertius*; however, *V. ovalis* can be distinguished from *V. spermatosphaera* by its larger gonidia, and from *V. tertius* by visible differences in gonidial chloroplast morphology.

AMPHIPODS EXCLUDE FILAMENTOUS ALGAE FROM THE WESTERN ANTARCTIC PENINSULA BENTHOS: EXPERIMENTAL EVIDENCE

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Hard bottom, subtidal communities along the western Antarctic Peninsula are dominated by forests of large, chemically defended macroalgae which support a very dense assemblage of amphipods. Free-living filamentous algae are rare in the subtidal, but filamentous algal endophytes are common in many of the larger macroalgae, both likely as the result of amphipod grazing pressure. Filamentous algae are common in the intertidal, but primarily in the upper intertidal and on high-energy shores where amphipods are likely to be excluded much of the time. We tested the hypothesis that free-living, filamentous algae would be rapidly consumed if transplanted from the intertidal to subtidal. The filamentous, intertidal green alga *Cladophora repens* was transplanted to the benthos in six different macroalgal habitats. Control algae were transplanted in 3 m deeper waters nearby (approximately 12 m or less laterally), but suspended 3 m off the bottom where amphipods are absent or rare. Overall consumption over approximately 6 h on the bottom ranged from 22 to 98% of the initial biomass while significantly less biomass loss occurred in the water column.

INTERACTIVE EFFECTS OF SHORE LEVEL AND EXPERIMENTAL WARMING ON ADULT AND JUVENILE INTERTIDAL KELP

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Predicted increases in temperature associated with global warming could have deleterious effects on intertidal organisms, the severity of which could depend on their vertical intertidal location. *Saccharina sessilis* is a dominant, intertidal, brown alga forming a consistent canopy in the low to mid intertidal zone. Forty permanent plots were manipulated to achieve levels of high (five adult plants) and low (single adult plant) canopy densities. Half of the canopy manipulations were also experimentally warmed during one low tide per month to raise ambient temperatures roughly 10 °C. Results for both juvenile recruitment and adult canopy cover indicated a weak positive effect of increasing intertidal height in the low zone, and a strong negative effect of increasing intertidal height in the high zone ($P < 0.001$, $P = 0.036$). Additionally, there was a significant Canopy Cover x Heat x Zonation interaction ($P = 0.019$) on juveniles, indicating that heating negatively affected recruitment in the high zone but only in low canopy cover plots. In this case, the effects of global warming were contextual, dependent upon both environmental (i.e. intertidal height) and biological (i.e. canopy cover) constraints.

IT'S NOT EASY BEING PINK: THE EFFECT OF INTERTIDAL STRESSORS ON CORALLINE ALGAL PHYSIOLOGY

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Coralline algae are central components of intertidal ecosystems, providing key ecological functions. At low tide, intertidal corallines experience a range of physiological stresses, such as light, temperature and desiccation stress, and differences in physiological tolerances likely play a role in habitat delineation. To quantify photosynthetic responses to abiotic stressors and explore putative survival strategies, physiological experiments were conducted on two species of coralline algae found in the Pacific Northwest, *Corallina vancouveriensis* (Yendo) and *Calliarthron tuberculosum* (Postel and Ruprecht) E.Y. Dawson. Photosynthesis was measured in submerged and emerged conditions, mimicking the course of a tidal cycle. Recovery after the application of intertidal stressors was also determined, mimicking the return of a tide. *Corallina* was found to be temperature tolerant when submerged in warm tidepools and capable of photosynthetic recovery after a low tide event. *Calliarthron* was sensitive to temperature increases, and not capable of recovering photosynthetically after a low tide event. Results from this study aid in understanding the physiological tolerances of these widespread coralline species to abiotic stressors.

LONG TERM STUDIES OF AN ARCTIC KELP COMMUNITY IN THE BEAUFORT SEA, ALASKA

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We examined benthic community structure and annual variations in kelp production in relation to physicochemical parameters, as well as biodiversity patterns, in the Boulder Patch, an isolated kelp bed community on the Alaskan Beaufort Sea coast. Long-term variations in kelp growth in coincidence with recent measurements of underwater photosynthetically active radiation, light attenuation coefficients, chlorophyll and total suspended solids, were measured to determine the impact of sediment resuspension on kelp productivity. Examination of a 30-year record of annual growth at two sites reveals other periods of low annual growth that are likely related to exceptional strong periods of storm activity. The exceptionally low growth of kelp in some years indicates that these plants are living near their physiological light limits, but represent excellent indicators of inter-annual changes in water transparency that result from variations in local climatology. Main algal biomass contributors were two species of red algae (*Phycodrys* and *Phyllophora*) and the brown alga *Laminaria solidungula*, and 141 invertebrate taxa, mainly sponges, bryozoans and hydrozoans. Many of these species may be good indicators for environmental conditions, and subsequently environmental change.

POPULATION GROWTH AND PLASTID TYPE OF *MYRIONECTA RUBRA* DEPEND ON THE KINDS OF AVAILABLE CRYPTOMONAD PREY

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Myrionecta rubra, a cosmopolitan red tide ciliate, is not only a predator on cryptomonads, but also is a main prey for harmful *Dinophysis* species. *M. rubra* seems to have plastid preference, which has been rarely studied using genetic analysis combined with growth rates. Population growth of '2-month starved' *M. rubra* strain MR-MAL01 was 0.09 per day when supplied with cryptomonad prey strain CR-MAL03, in sharp contrast to 0.48 per day with prey strain CR-MAL01. Both types of PCR product, 'CR-MAL03 type' and 'CR-MAL01 type' were continuously detected during 2 weeks of the second starvation in *M. rubra* cultures that had been grown with cryptomonad prey strain CR-MAL01 followed by the first starvation for 3 weeks before re-feeding with prey strain CR-MAL03 for 2 days. The *M. rubra* strain MR-MAL01 thus can ingest both prey strains, CR-MAL01 and CR-MAL03, and retain plastids of strain CR-MAL03 as well as CR-MAL01 for an extended period of time. TEM images of well-fed *M. rubra* were also analyzed to trace the ultra-structural dynamics of cell organelles inside the newly ingested preys. *M. rubra* may sometimes retain 'less-fit' plastids in spite of a resultant lower growth rate.

A PRELIMINARY REPORT ON GENETIC DIVERSITY OF THE MYTILID ENDOCOMMENSAL SCUTICOCILIATE *MYTILOPHILUS PACIFICAE*, INHABITING THE MUSSEL *MYTILUS CALIFORNIANUS*

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Antipa and Dolan (1985) described *Mytilophilus pacificae* as a new genus and species, endocommensal in the mussel *Mytilus californianus*. We collected ciliates from five mussel hosts at Pigeon Point and Pillar Point, CA, and Depoe Bay, OR, preserved them in 80% ethanol or Chelex, and sequenced for the rRNA gene region and cox-1 barcode following Strueder–Kypke and Lynn (2010). All sequences were identical for the rRNA gene region. Phylogenetic analyses of SSU rRNA placed *M. pacificae*, a representative of the family Peniculistomatidae, unambiguously in a cluster with species from the pleuronematid families Histiobalantidiidae and Pleuronematidae. Ciliates collected from two mussels at Pigeon Point and from one mussel at Pillar Point, ~30 km apart, had identical cox-1 barcode sequences. Ciliates from two mussels at Depoe Bay were 0.14% divergent from each other and 1.02% and 1.17% divergent in the barcode region from those at the two California localities, which are ~800 km from Depoe Bay. These very preliminary results suggest that there is genetic separation with distance. Supported by NSERC Canada.

AN EXAMPLE OF ALGAL CULTURE CONTAMINATION AND COLLAPSE IN AN OPEN POND

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Multiple laboratory and outdoor cultivation efforts with microalgae have shown that daily algal biomass productivity of 50 to 100 g dry weight/m^2/day is achievable, resulting in estimated annual production rates of 50 to 100 metric tons of dry algal biomass per acre. However, production from longer term (e.g. annual) efforts have achieved only 10% to 20% of that obtained over short-term intervals. Contamination is one factor that may be responsible for reduced yield and culture collapse. Information regarding culprit organisms and biotic and abiotic factors that affect population dynamics is limited. Population dynamics of the cultivated alga, *Scenedesmus* spp. and multiple contaminating species of zooplankton, including rotifers, ciliates, and amoebae were monitored. The occurrence, diversity and density of contaminating organisms increased with rising

temperatures from winter (5~15 C) to summer (15~38 C). From initial detection of grazing organisms to complete collapse of the *Scenedesmus* culture occurred within days. An assessment of grazing capacity of the major contaminating species revealed that rotifers were the greatest threat to *Scenedesmus* cultures, followed by *Vorticella* and amoebae.

TEMPORAL AND SPATIAL POPULATION GENETIC STRUCTURE OF THE RAPHIDOPHYTE *GONYOSTOMUM SEMEN*

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The bloom-forming raphidophyte *Gonyostomum semen* (Raphidophyte) has spread and become more abundant in Scandinavian lakes during the last 30 years and is now considered a noxious and invasive species. We investigated the population structure and genetic diversity over both temporal (one lake during 2 years, two blooms with samples collected every 3 weeks) and spatial (15 lakes in Scandinavia) scale of *G. semen* using Amplified Fragment Length Polymorphism (AFLP). On the temporal scale, increase of genetic diversity during a bloom and significant genetic differentiation between the two blooms were observed. The differentiation between the 2 years was likely due to genetic recombination during sexual reproduction at the end of the bloom resulting in resting cyst formation. The cysts provide new genotypes that germinate the subsequent year. Also, an increase of genetic diversity with time was observed within the bloom. In a second study we are investigating gene flow among lakes at small (a few km) and large scale (1000 km) and to identify the potential invasion pattern of *G. semen.*

STRAIN-SPECIFIC FUNCTIONAL AND NUMERICAL RESPONSES ARE BOTH NEEDED TO EVALUATE PREDATOR-PREY DYNAMICS: A CASE STUDY USING MODEL PROTOZOA

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We address two, inexorably linked, issues: (1) appreciating temperature-induced strain variation enhances model robustness; (2) incorporating both ingestion and growth functions alters predator-prey dynamics (cf. Lotka-Volterra approaches that assume constant assimilation). We evaluate these issues using the model dinoflagellate *Oxyrrhis marina* consuming the flagellate *Dunaliella primolecta.* Strain variation occurred across 10 *O. marina* isolates; maximum growth was determined at 10, 20, 26, 30 C, with variation revealed by ANOVA. Functional and numerical responses for two strains at 20 and 26 C revealed functional response alone cannot identify strain differences; i.e. numerical responses differed when functional responses did not. To explore intraspecific differences over a climate-shift scenario (6 C), responses were used to parameterise a predator-prey model. We conclude: (1) protozoan strain-differences must be appreciated when evaluating population dynamics; and (2) the standard method of modeling population dynamics can produce less accurate conclusions than those that include independently derived functional and numerical responses.

PREY DEPENDENT MORTALITY RATES IN MICROBIAL POPULATION MODELS

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Protozoa are key components of a wide range of ecosystems, but ecological models that incorporate these microbes often suffer from poor parameterisation, specifically of top-level predator loss rates. We (1) suggest that top-level predator mortality is prey-dependent; (2) provide a novel approach to assess this response; and (3) illustrate the ecological relevance of these findings. Ciliates, *Paramecium caudatum* (prey) and *Didinium nasutum* (predator), were used to evaluate predator mortality at varying prey levels. Data were used to determine non-linear mortality and growth responses over a range of prey abundances. The responses, plus literature data were then used to parameterise a predator–prey model, based on the Rosenzweig–MacArthur structure. Our method to determine variable mortality rate revealed a strong concave decline in mortality with increasing prey abundance. The model indicated average mortality rate superficially produced dynamics similar to the variable rate, but there were differences in the period of predator–prey cycles, and the lowest abundance of prey and predators (by ~2 orders of magnitude). The differences between incorporating variable and constant mortality rate indicate that including a variable rate could substantially improve microbial-based ecological models.

MULTIGENE PHYLOGENY OF THE BATRACHOSPERMALES (RHODOPHYTA)

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A phylogenetic inference of the strictly freshwater order Batrachospermales was reconstructed via multigene Bayesian analysis. This tree was based on two plastid encoded DNA markers *rbc*L, 1282 bps and UPA, 370 bps) and a mitochondrial marker (COI, 1232 bps). The Batrachospermales were monophyletic with strong statistical support. Our tree topology was similar to previously published single gene phylogenies. However, in our multigene tree most nodes were well supported, a result that was not previously achieved. Therefore, recent taxonomic changes within the order were substantiated. Endemic species of the ''Australasian Group'', formed a well-supported clade and it was clear that the cosmopolitan section *Setacea* is within this clade. Past analyses showed the morphologically similar subsections *Macrospora* and *Aristata* as separate clades, and our results confirm them as distant lineages. Although sections *Aristata*, *Acarposporophytum*, and *Turfosum* were hypothesized to be a clade in previous research, the current data provide statistical support to this hypothesis. Overall, our phylogeny inferred a similar tree topology to previously published analyses, but provides statistically supported insights into the relationships among sections for future taxonomic changes.

NEW TAXONOMIC CONCEPTS IN THE GENERA OF THE PEYSSONNELIACEAE (PEYSSONNELIALES, RHODOPHYTA) WITH FOCUS ON TAXA FROM THE GULF OF MEXICO AND PANAMA

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Post-oil spill impacts of the anthropogenic April 2010 BP Deepwater Horizon crude oil spill resulted in a dramatic decrease of seaweed diversity offshore Louisiana, Mississippi and Alabama in the northwestern Gulf of Mexico. The few taxa that were dredged in these areas in December 2010 and April 2011 were crust-forming members of the Peyssonneliaceae, i.e. *Peyssonnelia inamoena* and *Riquetophycus* spp. The generic concepts of these taxa will be critically assessed in a worldwide phylogeny of the family on the basis of comparative chloroplast-encoded *rbcL* and nuclear LSU rDNA sequence analysis. Diagnostic vegetative and reproductive morphological characters of other new taxa of Peyssonneliaceae from the Gulf of Mexico and Panama will be highlighted as well.

COMPARATIVE PHYLOGENY OF THE MIXOTROPHIC TESTATE AMOEBA *HYALOSPHENIA PAPILIO* LEIDY AND ITS ''ZOOCHLORELLA'' ENDOSYMBIONT (TREBOUXIOPHYCEAE)

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Arcellinid testate amoebae are free-living unicellular eukaryotes that play important roles in terrestrial and aquatic ecosystems. They are increasingly used as bioindicators to monitor a wide range of environmental changes, from global warming to pollution. Also they are commonly used as palaeoenvironmental indicators. However, the taxonomy and phylogenetic relationships among these organisms are still poorly known. *Hyalosphenia papilio* Leidy is a common mixotrophic testate amoeba in sphagnum peatlands. The nature and specificity of the relationship between the host (*Hyalosphenia papilio*) and their endosymbiont is still unclear. Our work identified these symbionts as green algae relate to family Coccomyxaceae, which also comprises sea anemone and lichen symbionts. We also reveal the degree of integration and co-evolution between the testate amoeba and the endosymbiotic algae.

POLYMER APPLICATIONS FOR IMPROVED ALGAE BIOFUEL PRODUCTION

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Algae biofuel has the potential to sustain global fuel demand beyond the capability of crop-based bioethanol, while maintaining environmental integrity.

Unfortunately, widespread adoption is hampered by cost-prohibitive areas of production, mainly due to the energy demands of water transport. This project is motivated by the need for improved algae biofuel economics, with aims targeted at optimizing algae harvest and oil extraction. Towards these aims, low-cost polymers have been designed and tested for concentrating and dehydrating algae, direct synthesis of biodiesel, and for direct extraction of oil from algae without contaminating the residual biomass with solvents. Algae can be harvested out of dilute suspension by ionic polymers that bind algae in a pH-dependent manner. Resin-bound algae can be easily dehydrated for efficient biodiesel synthesis. Hydrophobic resins can selectively accumulate neutral lipids out of lysed algae suspension, allowing for separation from residual biomass. In support of resin development, we have also developed rapid and accurate quantitative methods to assay algae biomass weight and lipid content from growth through extraction that are suitable for obtaining mass balances.

CHARACTERIZATION OF GENETICALLY ENGINEERED *SYNECHOCOCCUS ELONGATUS* PCC 7942 FOR BIOFUEL PRODUCTION

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In this work, a model cyanobacterium, *Synechococcus elongatus* PCC 7942, was genetically engineered for the production of free fatty acids (FFA), a precursor of biodiesel. While up to 57 mg/L of FFA was secreted from the engineered strains, growth was found to be impaired. Further characterization revealed additional changes including decreased chlorophyll a content, increased levels of phycobiliproteins, reduced photosynthetic yields, and lower rates of oxygen evolution. Hyperspectral fluorescence confocal microscopy revealed changes in the spatial ordering of the photosynthetic pigments, namely aggregation of pigments at the cell poles. This suggests either a physical change in the structure of the thylakoid membrane or reduced binding affinity of phycobilisomes to the thylakoid membrane. The underlying cause of these physiological effects was investigated by adding exogenous FFA to the cyanobacterial cultures and measuring the level of lipid peroxidation during FFA biosynthesis. The physiological effects analyzed in this study will ultimately limit FFA production and prevent continuous cultivation for large-scale processing. Hence, future work will focus on the use of novel enzymes to control FFA composition and investigation of alternative hydrocarbon products.

LIPID PRODUCTION AND PHOTOSYNTHETIC PERFORMANCE AS KEY CHARACTERISTICS TO ANALYZE NITROGEN STRESS-RELATED CHANGES IN TWO MARINE MICROALGAE

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The interest in the production of microalgae as biofuel is increasing due to their high oil content and rapid biomass production. Photosynthetic performance, lipid production and metabolic changes of two marine microalgae, *Dunaliella tertiolecta* and *Thalassiosira pseudonana*, in responses to different nitrogen (N) availability were monitored in this study. *D. tertiolecta* and *T. pseudonana* were incubated in N-replete medium till the stationary phase achieved and were transferred to N-depleted medium during 15 and 11 days periods, respectively. Responding to N depletion, growth rates of *D. tertiolecta* and *T. pseudonana* declined from initial values of 0.58 ± 0.11/d and 0.82 ± 0.04/d, respectively, to nearly zero on Day 2 or Day 3. From the spectrum results of Fourier transform infrared micro-spectroscopy (FTIR), the relative proteins and lipids contents varied in parallel with nitrogen limitation, but carbohydrates levels did not be affected. Fv/Fm and σPSII values measured by FIRe were also affected by N availability in both species. The combination results of photosynthetic per formance indices and biomass composition monitoring showed evidence that the metabolic strategies of microalgae to acclimate N limitation stress are species-specific.

NATURAL FRESHWATER PERIPHYTIC ALGAL COMMUNITIES FOR COMBINED WASTEWATER REMEDIATION AND CELLULOSIC BIOFUEL FEEDSTOCK PRODUCTION

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The anthropomorphic introduction of wastewater effluents rich in P and other mineral nutrients into natural aquatic communities is responsible for growth of harmful algal blooms and their impacts, which include coastal dead zones and animal poisonings. We evaluated the potential for natural periphyton communities collected from Lake Mendota, Dane Co., WI, to remediate wastewater by removing phosphorus. We also determined the cellulosic content of dry periphyton biomass harvested weekly through two growing seasons, June through November. We hydrolyzed extracted algal cellulose for use in cultivating bacteria that had been genetically modified to produce fatty acids suitable for biofuel production. Our results show that natural periphyton effectively removes wastewater P to below 0.005 mg per liter; that the readily-harvested, allomorph 1α-rich cellulosic crop reaches as much as 40% of community dry weight; and that cellulose extracted from such communities is a potential carbon source for the production of biofuels or other microbially synthesized compounds. We propose that periphyton communities cultivated in municipal wastewaters could readily be harvested for cellulose extraction for diverse applications, including biofuel feedstocks.

ECOLOGICAL RISKS OF GENETICALLY MODIFIED ALGAE IN COMMODITY-SCALE CULTIVATION

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Algae are being investigated as a potentially major contributor feedstock for renewable biofuels. Much of the research on algal biofuels involves genetic engineering of numerous genes involving various aspects of metabolism. In effect, the goal is to grow genetically modified (GM) algae at the commodity scale by mimicking some of the characteristics of ecosystem disruptive algal blooms (EDAB), that is, persistent monoculture of one organism. In addition to the obvious benefits, algal GM monoculture has the same types of potential environmental concerns as GM agricultural crops. What is the potential for survival, outbreeding and horizontal gene transfer of mass cultured GM algae should they escape into natural ecosystems? Most importantly, what is the incremental risk compared to mass culture of wild type algae, and what are the most appropriate assessment endpoints and biocontainment options?

EFFECT OF LIGHT QUALITY ON THE GROWTH AND LIPID CONTENT OF *CHLORELLA VULGARIS* BEIJ. (CV1) (CHLOROPHYTA)

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The Philippine isolate of a freshwater green alga, *Chlorella vulgaris* Beij. (CV1), was cultured under three different light quality: blue, red and white, using light emitting diodes (LED) and cellophane papers. LED experiment was conducted indoors with 2% CO_2 in air and under an average photon flux density (pfd) of 80 $\mu mol/m^2/s$ for 156 h (6.5/day), and 12:12 h, Light: Dark cycles. While the cellophane experiment was done outdoors under an average pfd of 2056 $\mu mol/m^2/s$, bubbled with air for 312 h (13day), and 8:16 h Light: Dark cycle. The alga showed the same trend in growth for the LED and cellophane experiments, i.e. cultures under blue light had the highest growth, followed in decreasing order by red and white lights. Both experiments showed

that of the 36 fatty acids, the only abundant saturated fatty acid is palmitic acid, which is a good source of biodiesel. However, the amount of palmitic acid was about 2× greater in the cellophane experiment than in the LED experiment, regardless of colors. It seems that pfd, photoperiod and duration of growth played a greater role in the fatty acid composition of the alga over the different colors.

A NOVEL, RAPID, SUB-MICROSCALE IN-SITU FATTY ACID ASSAY AND APPLICATIONS TO AQUATIC MICROORGANISMS

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The study of microalgal lipids has exploded in recent years driven by the increased interest in algal biofuel and nutraceutical production. A key limitation in traditional chemical techniques for GC/MS fatty acid analysis is the need for large amounts of algal biomass. To circumvent this problem, we developed a rapid, sub-microscale, *in-situ*, technique (SMIS) for acid-catalyzed transesterification. Using this method, quantitative lipid GC/MS analysis can be performed on minute samples (250 μg dry cell biomass), with approximately 5% variation. The method uses 5 to 25 times less solvent and reagent than the AOAC or AOCS recommended protocols. To demonstrate the broad application of the SMIS technique for assessing algal lipid profiles, we assayed over 60 algal species representing a broad range of taxa (e.g. Haptophytes, Crysophytes, Raphidophytes). The application of this technique to growth studies was achieved by measuring lipid profiles in *Chrysochromulina* spp., when the organism was exposed to varying physiological conditions (including light, pH, temperature, waste water), over time, using small volume cultures. Fatty acids of *Daphnia magnus*, *Drosophila melanogaster*, and *Brachionus calyciflorus* were also measured.

PHOTOSYNTHETIC ALVEOLATES AND THE EVOLUTION OF APICOMLEXAN PARASITES

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The origins of plastids in apicomplexans and dinoflagellates have constituted a central problem to our understanding of plastid endosymbiosis in eukaryotes. The major obstacle with these plastids have been their greatly reduced and divergent genomes, rendering comparison to other plastids and each other extremely difficult. This led to several mutually incompatible theories on their ancestry from green algal, red algal, or secondary red plastids. Recently, we presented data from two novel alveolate lineages, *Chromera velia*, and a mysterious Alveolate CCMP3155, which provide multiple lines of sequential and non-sequential evidence that the extant apicomplexan and dinoflagellate plastids share a common evolutionary origin, and are related to the plastid of heterokont algae. By investigating nucleus-encoded plastid-targeted genes, we extend this evidence and argue for multiple losses of photosynthesis and several possible losses of plastids in alveolates. To further support this, we report a new environmental plastid-bearing lineage branching between *Chromera* and apicomplexans that is closely associated with reef corals. We provide data on distribution of *Chromera*, CCMP3155, as well as the diversity of colpodellids, and discuss the evolutionary path of parasitism acquisition in apicomplexans.

THE FUTURE OF MICROALGAL TAXONOMY

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The urgent need to understand complex, global phenomena and the emergence of improved data management technologies are driving a transformation in biology that will enhance data-driven discovery. While taxonomy remains a parochial discipline with individual taxonomists working in isolation of each other and publishing their work in sometimes hard-to-obtain, printed journals, taxonomy can play a key role in this transformation and benefit from it. Taxonomic intelligence and a Global Names Architecture that use names, classifications and phylogenies to index data is emerging as a powerful device to organize information for all organisms. Natural Language Processing tools will extract information from the literature, and additional ontological frameworks will add to existing ones to model our knowledge.

We need interfaces through which scientists can improve the data pool, changes in work flows to utilize new technologies, tools to mobilize standards-compliant data at the point of origin, and incentives to promote participation in this process. Priorities for alpha taxonomy in phycology include the digitization of the phycological literature, annotation of taxonomic treatments and a further investment in and integration of existing algal resources.

A TYPE-2 ACYL-COA:DIACYLGLYCEROL ACYLTRANSFERASE GENE IS ESSENTIAL FOR ENDOPLASMIC RETICULUM-BASED TRIACYLGLYCEROL SYNTHESIS IN *CHLAMYDOMONAS REINHARDTII*

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Triacylglycerol (TAG) produced by microalgae is a promising feedstock for biofuels, but is low in naturally occurring stains. Over production of TAG through genetic and metabolic engineering of microalgae has resulted in limited improvement due to inadequate understanding of the molecular and cellular mechanisms underlying TAG formation. The genes involved in TAG synthesis in a wild type and a TAG hyper-accumulation mutant of *Chlamydomonas reinhardtii* were studied by a genetics, transcriptomics, proteomics and lipidmics approach. Of multiple genes investigated, DGAT2, which encodes diacylglycerol acyltransferase (DGAT) was most sensitive to environmental stimuli inducing TAG synthesis. Application of transcriptional and translational inhibitors showed that the DGAT2 gene was regulated at the transcriptional level. Artificial microRNA mediated silencing of DGAT2 resulted in reduction in several endoplasmic reticulum (ER)-derived TAG species, confirming that DGAT2 is essential for ER-based TAG synthesis. Accumulation of plastid-derived TAG species was observed in the DGAT2 mutant relative to the wild type, suggesting that the TAG synthesis pathway in the chloroplast can compensate for the reduction in TAG synthesis in ER, thereby maintaining lipid and energy homeostasis in stressed cells.

PHOSPHOLIPID:DIACYLGLYCEROL ACYLTRANSFERASE IS INVOLVED IN LIPID SYNTHESIS AND DEGRADATION IN *CHLAMYDOMONAS REINHARDTII*

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Many microalgae, under stress, store carbon and energy in the form of triacylglycerols (TAG). The molecular and cellular mechanisms for TAG synthesis and storage are poorly understood. We obtained a full-length cDNA encoding a putative phospholipid:diacylglycerol acyltransferase homolog (PDAT) that catalyzes conversion of phospholipids and diacylglycerol (DAG) into TAG. In silico analysis revealed that the PDAT gene has homology with mammalian lecithin:cholesterol acyltransferases (LCAT), which also has a typical lipase motif (G/AXSXG), a characteristic of hydrolytic enzymes. Transcriptional analysis of PDAT and glycerolipid profiling of Chlamydomonas cells indicated that PDAT is a housekeeping pathway responsible for phospholipid turnover while maintaining a basal level of TAG under normal growth conditions. The PDAT pathway is up-regulated and thus contributes to the overall accumulation of TAG production under stress. In vitro and in vivo enzyme assays showed that PDAT contributes to TAG synthesis through two pathways: (1) transacylation of DAG with acyl groups from various species of phospholipids; and (2) DAG:DAG transacylation to form TAG and monoacylglycerol. Lipolytic acyl hydrolase activities of PDAT were demonstrated with TAG, phospholipids, galactolipids, and cholesterylesters as substrates.

A TALE OF TWO SEAS: THE ATLANTIC-MEDITERRANEAN TRANSITION IMPOSES A STRONG GENETIC BREAK IN THE PROTIST *OXYRRHIS MARINA*

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Biogeography remains fundamental to evolutionary biology as understanding spatiotemporal distributions, dispersal, and geneflow offers insight into speciation and extinction processes. Arguably, our understanding of the biogeography of free-living unicellular organisms remains relatively poor, and only recently has research moved beyond over-generalisations about biogeographic patterns to characterising demography and assessing the role of landscape in shaping distributions. Here we examine the distribution and diversity of the marine flagellate *Oxyrrhis marina*, a re-emerging ecological and genetic model organism, across the North East Atlantic (NEA) and Mediterranean Sea (MED). Based on analysis of three genes for >200 isolates we demonstrate a conspicuous phylogeographic break in the *O. marina* distribution corresponding to the NEA/MED transition. Different clades occupied NEA and MED coastlines and haplotype diversity was conspicuously higher within the MED. Genetic diversity patterns and molecular dating estimates suggest a northward expansion, and subsequent divergence, of *O. marina* from a historical Mediterranean population, likely in the late Pliocene/early Pleistocene. The intriguing question of how such historical signals are maintained in highly dispersive organisms in a high connectively marine environment remain to be addressed.

POSTERS

A STUDY OF SOFT-ALGAE ASSEMBLAGES AND THEIR CORRESPONDENCE WITH DIATOM INDICATOR SPECIES WITHIN U.S. RIVERS AND STREAMS – PRELIMINARY FINDINGS

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Diatoms have been widely used as environmental indicators to assess water quality. In contrast other algae present, sometimes referred to as soft-algae, are used relatively little. This study uses data from the EPA''s recent National Rivers and Streams Assessment to compare the soft-algae assemblages of samples containing selected diatom species to evaluate the use of soft-algae in water quality assessment, and to try and identify reliable indicator species. Data for comparison were identified using over 30 diatom indicators associated with five different environmental conditions. Initial findings have revealed particular soft-algae genera that may prove useful for assessment. However, specific species have yet to be identified. Difficulties in species level identification for soft-algae are considered a major contributing factor to these difficulties. Additionally, comparison of the two count methods used in the project suggests standard count methods be revised. Results show some congruence with a previous evaluation of soft-algae as water-quality indicators for the USGS NAWQA project, and also other ecological information in the literature.

MICROBIOTIC SOIL CRUST ABUNDANCE AND COMMUNITY DIVERSITY VARIES ACCORDING TO MESOSCALE LANDFORM PATTERN IN THE MOJAVE DESERT

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Abundance, distribution, and coarse resolution biodiversity of microbiotic crust were investigated on a piedmont landscape in the Clark Mountain Wilderness area, Mojave Desert. In our study area we characterized seven mesoscale landform units as: bar, swale, flattened bar, flattened swale, bioturbated unit, desert pavement, and shrub zone. We predicted that the seven landform units would have distinct microbiotic crust community characteristics. On each landform unit, microbiotic crust distribution was inventoried using cover and frequency quadrats, coarse resolution biodiversity was assessed on composite soil surface samples using the moisten soil method, and biomass was measured using chlorophyll-a extraction. Our results indicated that landform pattern had a major effect on microbiotic crust characteristics. Out of the seven landform units, the bar had the greatest crust cover, morphological/functional group richness, highest amount and diversity of dominant algae, and biomass. This unit is characterized by highest microtopography, which may enhance water infiltration, trapping of fine particles, and microclimatic conditions. Overall, our data indicate that microbiotic crusts are not randomly distributed and that some landform types are more suitable habitats for crust development.

DISTRIBUTIONS OF DIATOMS IN THE COASTAL WETLANDS OF LOUISIANA AND THEIR POTENTIAL USE AS SEA-LEVEL INDICATORS

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Sea level is an important driving force behind coastal wetland change, and predictions of future

rising sea-level rates have amplified concerns over wetland sustainability. To better understand the impact of future sea-level scenarios, recent past sea-level trends and the influence of sea level on marsh-building processes must be considered. Microfossils of diatom algae can be a powerful tool for sea-level reconstructions because diatoms are sensitive to environmental conditions such as marsh elevation, respond rapidly to environmental change, and have a silica frustule that preserves well within most sediments. We examined diatom species composition from surface sediment samples and their distribution in relation to environmental parameters, and determined the value of diatom transfer functions for reconstructing late Holocene sea-level changes in coastal wetlands of Louisiana.

ANALYSIS OF STRUCTURE AND COMMUNITY COMPOSITION OF EPILITHIC ALGAL-BASED BIOFILMS ON LIMESTONE BUILDING MATERIALS

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Epilithic microbial biofilms contribute to vital ecosystem processes such as nutrient cycling, biodiversity and primary productivity but also cause biodegradation, posing a threat to building structures and historic monuments. This study is an analysis of community composition and structure of biofilms colonizing limestone building materials recently quarried in Central Texas. Polished block sectioning techniques and scanning electron microscopy with backscatter detection (SEM-BSD) reveal biofilms range in thickness from 2 µm to 30 µm and inherent organisms are not uniformly distributed. Biofilms generally accumulate on amorphous substrates overlying limestone and in limestone cavities. This selective biofilm growth may be due to niche spaces created within limestone that retain water and adsorb limiting nutrients. Community composition was analyzed using molecular and morphological identification techniques; the major taxa of algae include representatives from Chlorophyceae, Trebouxiaphyceae, Bacillariophyceae, Oscilatoriales, and Pseudanabaenales. Diatoms occupy the upper-most layer of thick biofilms whereas cyanobacteria and green algae are ubiquitous inhabitants of lower layers. Future assessment of initiation and succession within the biofilm community will identify keystone micro-organisms.

A MODIFIED METHOD FOR QUANTIFYING STREAM-INHABITING, NON-DIATOM BENTHIC ALGAE

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A modified quantitative method for non-diatom benthic algae was applied in two large stream biomonitoring projects in California. Algal species identification and biovolume estimation were improved by separate processing of macroalgal and microalgal fractions. We analyzed data from 104 streams, classified into three categories according to the degree of human influence. A total of 260 non-diatom benthic algal taxa were recorded and mean algal biomass per site was 70.2 mm^3/cm^2 (range: 0.00003 – 836 mm^3/cm^2). Algal biomass obtained by the quantitative method was strongly positively correlated with field filamentous macroalgal cover and chl a concentrations. Analysis of similarity performed on each of three data sets: species biomass, species presence-absence, and proportions of taxonomic groups consistently showed benthic algal community composition was significantly different between high- and low-influenced sites. Of environmental variables tested, conductivity, dissolved organic carbon, sulfate, TDN, and TDP were the water-chemistry constituents exhibiting the strongest relationships with algal community composition based on nonmetric multidimensional scaling ordination. *Nostoc verrucosum* was an indicator of low TDN, while *Cladophora glomerata* and *Rhizoclonium hieroglyphicum* were indicators of high TDN.

SEASONAL CHANGE IN THE COMPOSITION OF MICROALGAE IN ABUKUMA RIVER, FUKUSHIMA

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Several cities are located along Abukuma River in Fukushima, causing high BOD and turbidity compared to other major rivers in Tohoku region. Nutrient and chlorophyll a concentration, turbidity, cell count using net (41, 20, 10, 5 µm opening) or filtered (GF/F, 0.7 µm) sample for flowing water were

conducted weekly at Kuroiwa in Fukushima city from October 2008. Inorganic nutrients were high from fall through winter. This seems to correspond to chlorophyll a concentration. Highest chl. a concentration was found in spring. Benthic diatoms were abundant from autumn through spring and cyanobacteria increased in summer. The particles sized 0.7 – 5 μm contributed more than 50% of the total turbidity. Bimonthly – seasonal sampling at several points through the river suggested inflow of nutrients and chl. a from tributaries in Koriyama city. Decrease in benthic algae, increase in planktonic algae, decrease in dissolved nutrients were found in one of the two reservoirs in the main river between Koriyama and Fukushima. Benthic diatoms dominated in the flowing water sample in downriver from the dam.

AN INVENTORY OF THE ALGAE OF HARRIMAN AND BEAR MOUNTAIN STATE PARKS, NEW YORK

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Harriman and Bear Mountain State Parks are just 30 miles from New York City. Nonetheless the parks contain a rich algal flora. During studies over three consecutive years (2008–2010), we encountered 371 species of freshwater algae (excluding diatoms and a cyanobacteria). Most of these were new records for Harriman State Park and many are new records for the State of New York. Using these samples we investigated the relative contribution of different collection strategies to our knowledge of the regional algal flora. Because many of the species encountered in the park are considered rare or infrequent, we investigated the occurrence and distribution of many species to determine why they might appear to be rare. We conclude that many of the species are present in low abundance. Additional sampling will be needed to completely document the algal flora.

THE DIATOM FLORA OF THE SOILS OF THE ATACAMA DESERT, CHILE

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The Atacama Desert in coastal Chile is the driest hot desert in the world. We collected a total of 88 soils samples from a 1000 km long region of the Atacama (La Serena to Iquique) as part of a study of the algal flora of the soils of the Atacama Desert, and here report on the diatom portion of that flora. Most of the soil samples collected contained no recoverable chlorophyte or cyanobacterial taxa. Thirty-two of the samples had at least one green algal or cyanobacterial isolate; these samples were examined for diatom frustules. A total of 49 different diatom taxa were recovered from the soils. Of these, the clear soil inhabitants were *Hantschia amphioxys*, *Luticola cohnii*, *L. goeppertiniana*, *L. mutica*, *L. nivalis*, *L. ventricosa*, *Pinnularia borealis*, and *Pinnularia subcapitata*. There were several of other taxa reported from desert soils previously, including *Epithemia adnata*, *Denticula valida*, and *Nitzschia valdecostata*. *Planothidium lanceolatum*, a generally aquatic taxon, was also fairly common. A number of other aquatic taxa were present in very rare numbers, and we suspect wind-blown transport for these species.

DOCUMENTING THE DIVERSITY OF AMOEBAE

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Although modern molecular methods have successfully started to uncover relationships between the main lineages of amoeboid organisms, the morphological diversity, geographic distribution and natural history of amoebae remain poorly known. This phenomenon is caused in part because limited morphological characters and concise monographs with good illustrations are not available for analyses. We are using both morphological and molecular tools to characterize both testate and naked amoebae from two local freshwater sites: the Smith College Greenhouse and a freshwater bog. At these sites, we see a wide range of amoebae, some of which we have been able to culture. We are now documenting this diversity first using light microscopy and SEM and then, for a more limited number of cultures, by PCR. These studies will contribute to the growing body of knowledge on the phylogeny and biogeography of free-living amoebae.

SUMMER PHYTOPLANKTON DIVERSITY IN 45 LAKES IN THE PUGET SOUND REGION OF NORTHWEST WASHINGTON

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We sampled forty-five lakes in the Puget Sound region of Northwest Washington to investigate the

relationship between water quality, site characteristics, and algal population structure. Water samples were collected each summer from 2006–2010 to measure nutrients (total phosphorus, soluble orthophosphate, total nitrogen, ammonium, and nitrate/nitrite), alkalinity, chlorophyll a, dissolved oxygen, specific conductance, and temperature. Watershed characteristics were recorded to assess shoreline composition and dominant land use. Phytoplankton samples were collected, preserved, and concentrated in settling chambers to determine taxonomic composition and algal biovolume. Unpreserved phytoplankton samples were also collected and used to generate a species list for each lake. The data were examined using correlation analysis, clustering, and principal components ordination to evaluate relationships between water quality parameters and phytoplankton assemblages. As expected, there was a strong correlation between total phosphorus and chlorophyll-a, and the lakes could be clustered based on similarity in water quality parameters. The phytoplankton communities were quite diverse, and many species were collected that are described as uncommon in the taxonomic literature. Lakes with low chlorophyll concentrations generally had a very diverse algal community.

QUANTIFICATION OF HAPTOPHYTE SPECIES BY PYROSEQUENCING: IS READ ABUNDANCE PROPORTIONAL TO CELL NUMBERS?

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Amplicon-sequencing of ribosomal RNA genes has become a standard for assessing microbial diversity in environmental samples. Abundance is of interest in addition to presence/absence of species to assess their ecological importance, but the proportion of reads from a particular species may not reflect the proportion of the species in a sample in terms of cell number. We investigated the relationship between cell number and the number of reads appearing in a sequence library constructed from an artificial community of haptophytes, and we tested whether there was a correlation between read abundance and cell size. One million cells from each of 11 haptophyte species were pooled, nucleic acids extracted, and the V4 region of the small ribosomal subunit gene was amplified. The amplicons were sequenced by 454-pyrosequencing. Our results show that the proportion of reads from the different species in the library was significantly different from the proportions of cells in the pool. There was a positive correlation between cell volume and read abundance, and thus cell size should be taken into account when interpreting 454-sequence data quantitatively.

THE EFFECTS OF SEASON AND SYMBIONT ON LIPID CONTENT OF THE INTERTIDAL ANEMONE *ANTHOPLEURA ELEGANTISSIMA*

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Anthopleura elegantissima, a common Pacific sea anemone, can host at least two endosymbionts: zooxanthellae (*Symbiodinium muscatinei*) and zoochlorellae (*Elliptochloris marina*). The symbionts of *A. elegantissima* provide photosynthetic carbon to supplement the host's heterotrophic feeding. Zooxanthellae potentially translocate five times more carbon to the host than do zoochlorellae. Exploring lipid levels of anemones in different symbiotic states can clarify whether more translocated carbon equates to more energy available for growth and reproduction. After extracting the lipids from the anemones, the lipids are separated into classes with thin layer chromatography (TLC). The lipid separation allows us to determine how the lipids differ based on season, symbiont, and reproductive condition of the *A. elegantissima.* By measuring the lipids in green, brown, and asymbiotic anemones in summer and winter we can relate lipid levels to cycles of gametogenesis and reproduction, which will allow us to draw conclusions about the contribution of the symbionts to the fitness of the host anemone.

CARBONATE-BORING ALGAE: MORPHOLOGY IN CULTURE OF CELLS AND CASTS FROM TROPICAL AND TEMPERATE WATERS

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As the role of carbonate-boring algae in tropical reef ecosystems becomes both better understood and of greater concern in the contexts of anthropogenic global warming and ocean acidification, it becomes more important to understand the biodiversity of the algae and the contributions made by individual species to reef productivity and reef dissolution rates. Here, we report research based on a library of cultured carbonate-boring algal strains isolated predominantly from the tropical waters of Hawaii and the cold-temperate waters of Washington and Massachusetts. Strains of several taxa of cyanobacteria (*Hyella, Mastigocoleus,* ''*Plectonema*''), chlorophytes (Eu*gomontia, Gomontia, Ostreobium, Phaeophila, Ruthnielsenia*), and rhodophytes (*Porphyra*) that are well represented in calcareous substrata are in the collection. We assessed strains for their ability to bore into calcareous substrata and for their temperature tolerance, and prepared casts for direct comparison of boreholes with ichnotaxa from the literature. From morphological, physiological, and molecular data, we infer that the biodiversity is significantly greater than previously recognized, for all taxa but especially for green algae previously assigned to *Ostreobium quekettii* and *Phaeophila dendroides*.

THE EFFECT OF TEMPERATURE AND GROWTH RATE ON TEP PRODUCTION BY *THALASSIOSIRA WEISSFLOGII*

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Global surface ocean temperatures are increasing. *Thalassiosira weissflogii* was grown in semi-continuous culture in a sequence of temperature and dilution rate changes to test the hypothesis that temperature increase and growth rate change affect the release of transparent exopolymeric particle (TEP) by diatoms. It was found that the temperature and growth rate all influence TEP formation. The size of TEP increased with temperature or growth rate increase. However, TEP concentration decreased with increased size of TEP. Therefore, the areas of TEP showed in the sequence temperature or growth rate were closed. Carbohydrate concentration per cell increased linearly with rising temperature and part of positive correlated with growth rate. The aggregations of *T. weissflogii* were measured through monitoring the particle size distribution of cultures by a laser in situ scattering and transmissometry (LISST-100X) instrument. The results indicated that the aggregation of *T. weissflogii* increased with increasing temperature or increasing growth rate. And size of cell was bigger in the high growth rate than those in the low growth rate. These influences have a profound impact on biogeochemical cycling of carbon.

HAPLO-DIPLOIDY IN THE COCCOLITHOPHORE *EMILIANIA HUXLEYI* (PRYMNESIOPHYCEAE): A DEFENSE AGAINST THE MARINE CILIATE PREDATOR *STROMBIDINOPSIS ACUMINATUM*?

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Emiliania huxleyi possesses a haplo-diploid life cycle with a calcifying diploid phase and a flagellated, non-calcifying haploid phase, with both able to reproduce asexually and sexually. We are investigating a predator defense function for the *E. huxleyi* life cycle. Preliminary 24 h prey disappearance feeding experiments showed that calculated ciliate ingestion rates on haploid cells were lower in bottles with higher ciliate densities, suggesting that either (1) haploid growth rates were stimulated by some nutrient(s) present in the ciliate culture or (2) haploid cells have an inducible defense system against the ciliate that is activated at higher ciliate densities. Ingestion rates on diploid cells were not significantly different across ciliate densities. Separate experiments indicated that both phases were significantly stimulated by 1.0 μ ciliate culture filtrate, suggesting use of organic compounds (osmotrophy) or bacteria (phagotrophy) for growth. However, growth rate stimulation was not high enough to explain apparent decreased ciliate feeding rates on haploids at high ciliate densities. Analysis of an experiment investigating induction of an *E. huxleyi* defense system by the ciliate is underway.

THE TOXICITY OF POLYUNSATURATED ALDEHYDE-CONTAINING *SKELETONEMA* SPP. DIATOMS TO TWO CILIATE PREDATORS

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Polyunsaturated aldehydes (PUAs) produced by diatoms have been shown to affect copepods negatively, possibly constituting a defense against these grazers. However, microzooplankton sometimes consume more diatom production than copepods, so our goal was to test whether a PUA-based defense mechanism is effective against microzooplankton grazers. Species and strains of *Skeletonema* spp. were tested for PUA composition and content: PUA composition was similar across *Skeletonema* strains, while total PUA:C ratios ranged widely, from a low of 86 ppm to a high of 1401 ppm. Grazing experiments were conducted, and both *Strombidinopsis acuminatum* and *Favellaehrenbergii* ingested *Skeletonema* at rates that were low compared with non-diatom prey. *S. acuminatum* ingested significantly less of the high-PUA *Skeletonema* sp. than the other two *Skeletonema* spp. tested. An experiment testing the effects of filtrate from *Skeletonema* and the phytoflagellate *Isochrysis galbana* on *S. acuminatum* proved that the filtrate from diatoms was detrimental to ciliates. Review of our past experiments indicated that negative effects of *Skeletonema* on ciliates could be due to dissolved substances released by the diatoms.

GRAZING IMPACT OF HETEROTROPHIC DINOFLAGELLATES AND CILIATES ON COMMON RED-TIDE EUGLENOPHYTE *EUTREPTIELLA GYMNASTICA* IN MASAN BAY, KOREA

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To investigate the grazing impact of heterotrophic protists on *E. gymnastica*, we measured daily the abundances of *E. gymnastica* and co-occurring potential heterotrophic protistan grazers in Masan Bay, Korea, in August 2004. We tested whether the several common heterotrophic dinoflagellates and the naked ciliates were able to feed on *E. gymnastica*. We also measured their growth and ingestion rates on *E. gymnastica* as a function of prey concentration. Finally, we calculated the grazing coefficients by combining field data on the abundance of the heterotrophic dinoflagellate and ciliate grazers and co-occurring *E. gymnastica*. The maximum growth rate of *G. dominans* on *E. gymnastica* was higher than that of *O. marina* or *P. bipes*. However, *E. gymnastica* did not support positive growth of *P. kofoidii*, *Strobilidium* sp., and *Strombidinopsis* sp. The maximum ingestion rates of *G. dominans*, *P. kofoidii*, *P. bipes*, *O. marina*, and *Strobilidium* sp. on *E. gymnastica* were similar, but they were much lower than that of *Strombidinopsis* sp. As calculated grazing coefficients, 3% of *E. gymnastica* populations were removed by the population of each of these heterotrophic protistan grazers in 1 day. The results of the present study suggest that *P. bipes*, small heterotrophic *Gyrodinium* spp., and naked ciliates (< 50 mm in cell length) sometimes have considerable potential grazing impact on the populations of *E. gymnastica*.

UPTAKE OF CADMIUM BY *CHARA AUSTRALIS*: PROSPECTS FOR PHYTOREMEDIATION OF CONTAMINATED SEDIMENTS

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Cd contamination is a serious environmental problem globally. Phytoremediation, the use of plants to remove contamination, has been extensively tested using terrestrial plants to extract Cd from soils. The use of aquatic plants to extract Cd from waters has also been investigated, but little work has been done using macrophytes to remediate Cd-containing aquatic sediments. We investigated the potential usefulness of *Chara* for phytoremediation, using *C. australis* (R. Br.) as a representative Charophyte. *C. australis* survives up to 30 ppm Cd in the sediment. Histochemical staining showed that *C. australis* accumulates Cd in rhizoids, nodal cells, and intermodal cells in the walls and in the cytoplasm. Cd accumulation increases with soil concentration. At 8 ppm Cd, *Chara* shoots and rhizoids accumulated 14 ± 2.9 μg Cd/g dry weight DW and 15 ± 1.3 μg Cd/g DW, respectively. Shoots of plants exposed to 8 ppm Cd had significantly lower concentrations of glutathione (GSH) (194 ± 21 nmoles/g shoot DW) than control plants (380 ± 17 nmoles/g shoot DW, $P < 0.05$).

EFFECTS OF NITROGEN ON RATES OF PHOTOACCLIMATION BY INVASIVE AND NATIVE SPECIES OF *GRACILARIA* (RHODOPHYTA)

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Invasive marine algae are a major threat to coral reef ecosystems in Hawai'i. Identifying characteristics of algae that facilitate their invasiveness can contribute to preventing future introductions as well as aid in early identification of potential invaders. One characteristic that may be instrumental in invasiveness is a high relative rate of photoacclimation, the adjustment of the photosynthetic apparatus in response to changed irradiance. Rapid photoacclimation may increase the competitiveness of an organism. Prior studies find significant disparities between photoacclimation rates for invasive and native tropical macroalgae, but have not considered the contribution of nutrification, a common problem in nearshore ecosystems that provides essential macronutrients for photoacclimation. This project aims to elucidate the extent to which the speed of photoacclimation of invasive algae is influenced by elevated levels of nutrients. *Gracilaria salicornia*, an invasive, and *G. coronopifolia*, a native, will be cultured and acclimated to different irradiance regimes and exposed to ecologically relevant nitrogen concentrations. Preliminary results suggest that elevated nitrogen elicits photosynthetic responses similar to shade acclimation, with increased phycobilin concentrations.

NITROGENASE GENE EXPRESSION IN THE BENTHIC ALGAL COMMUNITIES FROM CALIFORNIAN STREAMS

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Nine Californian streams were sampled for benthic algae in the fall 2010 and RNA extractions were done shortly after collection from representative algal samples. The diatoms *Epithemia* and *Rhopalodia* containing endosymbiotic cyanobacteria, and free-living heterocystous cyanobacteria *Nostoc verrucosum* and *Calothrix* spp. were recorded in five of the streams at nitrate concentrations below 0.01 mg/L and expression of nitrogenase was detected by real-time PCR. In higher nutrient streams (0.15–11.2 mg/L nitrate) nitrogenase expression was not detected. In a related study, we tested whether nitrogenase expression in *N. verrucosum* was correlated to heterocyst frequency and ambient nutrient availability. Nitrogenase expression of *N. verrucosum* was quantified by real-time PCR relative to 16S rRNA activity using the 2-ΔΔct method. The frequency of heterocysts ranged from 1.8 to 5.9% of the cells for colonies of different physiological stages. The nitrogenase expression correlated positively with heterocyst frequency in largest colonies only. Both nitrogenase expression and heterocyst frequency in largest colonies correlated negatively with ambient nitrate and orthophosphate concentrations, although not significantly.

SURFACE AND SETTLEMENT CUES OF *VAUCHERIA LITOREA* AND THE ESTABLISHMENT OF THE *ELYSIA-VAUCHERIA* SYMBIOSIS

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The mollusk *Elysia chlorotica* has an obligate symbiotic relationship with marine species of *Vaucheria* (Xanthophyceae). *E. chlorotica* requires *Vaucheria* for metamorphosis of veliger larvae and for the acquisition of its chloroplasts (kleptoplasty). Specificity of algae for feeding and metamorphosis were investigated using bio- and settlement assays, while surface composition (glycans) of algae was investigated using confocal microscopy. Adult animals and veliger larvae showed clear preferences in these assays, and the surface composition of *Vaucheria* proved unique when compared to other morphologically similar algae. Culturing of *E. chlorotica* in our laboratory enabled investigation of the initial phases of kleptoplasty. *Vaucheria* chloroplasts were ingested within hours of settlement on *Vaucheria*, but were not permanently integrated into the animal until 5–7 days of continued feeding. Removal of the algal food prior to this window resulted in cessation of morphogenesis and animal death, while removal of algae after 7+ days of feeding resulted in chloroplast retention and continued development. This ''reversibility window'' is now our focus for differential patterns of gene regulation, host immune response, and understanding of kleptoplast function in the animal.

ALGAL TURF PRODUCTION ON THE GREAT WICOMICO RIVER, VA, USA: DIVERSITY AND BIOCHEMISTRY OF AN ALGAL TURF SCRUBBER (ATS)

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Algal Turf Scrubbers (ATS) are mini-ecosystems dominated by periphyton (algal turfs). Ranging from

0.1 to 3 hectares, ATS systems have operated for decades providing multiple benefits to aquatic environments, removing nutrients, injecting oxygen, and producing biomass for biofuels and co-products. Two experimental systems were implemented on Chesapeake Bay's Great Wicomico River to assess periphytic diversity, nutrient uptake/removal, and biochemical by-products. Additional experiments were conducted on one of these systems, with the injection of CO_2 and oxamate triggers to verify effects on community structure and biochemical output. Algal production on an annual cycle produced monthly means between 10–70 g (35 g avg.) dry weight/m^2/day. Diatoms (Bacillariophyta) dominated the systems: *Berkeleya rutilans, Melosira nummuloides*, and *M. moniliformis* common during warmer months and species of *Grammatophora* during colder months. The chlorophyte *Ulva intestinalis* was abundant in spring and early summer. Cyanobacteria, Rhodophyceae, and Chrysophyceae were also present. In one experiment with CO_2, having a significant drop in pH, cyanobacterial biomass (*Lyngbya* cf. *salina*) dominated the system. Omega-3 fatty acid increases during colder months; carbohydrates ranged from 20–25% of dry weight, with an ash content of 55–60%.

COMPETITIVE ASSAYS OF TWO MIXOTROPHS AND TWO DIATOMS FROM THE ROSS SEA, ANTARCTICA

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Mixotrophy, a combination of autotrophic and heterotrophic modes of nutrition, has potential to affect competitive interactions with strictly autotrophic organisms. For example, non-phagotrophic photosynthetic algae may to out-compete mixotrophic flagellates in low DOC environments, where autotrophy is the dominant C assimilation pathway. To test whether mixotrophs are competitively inferior to strict autotrophs under such conditions, we conducted competition experiments between four Antarctic algae: two diatoms – *Fragilariopsis* sp. and *Fragilaria* sp.; and two mixotrophic flagellates – *Geminigera cryophila* and *Chrysolepidomonas dendrolipdata*. Experiments were conducted in f/2 media made with artificial seawater (34 psu) with no added dissolved carbon. Contrary to expectation, the competitive outcomes were species dependent, with no overall advantage for mixotrophic flagellates or diatoms as a group. *G. cryophila* appeared to be the dominant competitor to the other three species under these conditions. These results may reflect differences in the relative reliance on phototrophy among the mixotrophic Antarctic algae, with *G. cryophila* more specialized in phototrophic activity than in heterotrophic energy gain. Mixotrophy may provide a range of competitive advantages for microbial organisms in the Southern Ocean.

RESPONSE OF THE EPIPHYTIC ALGAL COMMUNITY TO EXPERIMENTALLY ELEVATED NUTRIENT LEVELS IN THE GUANA TOLOMATO MATANZAS NATIONAL ESTUARINE RESEARCH RESERVE

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As anthropogenic impacts continue to escalate in waterways, nutrient additions may have profound impacts on the epiphytic algal community in coastal habitats. These increased nutrient loads may lead to drastic changes in the algal community present, with subsequent impacts on trophic level interactions with macrophytes and invertebrates. The purpose of this project is to assess the impacts of elevated levels of nitrogen and phosphorus on the epiphytes of *Spartina alterniflora*, a common macrophyte found in northeast Florida estuaries. Clay pots containing four separate nutrient treatments (0.5 M nitrogen, 0.5 M phosphorus, 0.5 M N + P, and control) with eight replicates infused with agar were placed at the base of *S. alterniflora* stands in a coastal estuary located within Guana Tolomato Matanzas National Estuarine Research Reserve (GTMNERR) in Ponte Vedra Beach, Florida. Epiphytic samples were collected on a monthly basis to examine changes in the epiphytic community caused by nutrient addition (chlorophyll *a*, ash-free dry mass and community composition as evidenced by microscopic investigation). Preliminary data indicate that chlorophyll *a* and AFDM levels are not significantly ($P > 0.05$) affected by the location within the estuary.

THE MARINE DINOFLAGELLATE GENUS *DINOPHYSIS* RETAINS PLASTIDS OF SEVERAL ALGAL ORIGINS

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The photosynthetic *Dinophysis* species are well known to have plastids of a cryptophyte origin, more specifically *Teleaulax amphioxeia*. Nonetheless, recent some molecular studies carried out with wild samples and laboratory culture strain reported that in addition to the plastids of *T. amphioxeia* origin, *Dinophysis* could also temporarily retain the other types of plastids. To investigate plastid diversity, we isolated a total of 66 phototrophic *Dinophysis* spp. cells between 2008 and 2009 from 8 sites along western and southern coasts of Republic of Korea and amplified psbA gene as a tracer from individually isolated cells and then digested the PCR products with a restriction enzyme, SfaNI. During this study, we often encountered ''green'' *D. acuminata* cells, which contained varying degree of red autofluorescencing green plastids within the cell, in addition to typical orange autofluorescencing plastids. The RFLP patterns revealed that a total of 66 *Dinophysis* cells analysed in this study all contained *T. amphioxeia*-type plastid. Further, we found that approximately two-thirds (~66%) of the analysed *Dinophysis* cells contained another cryptophyte plastid (i.e. *T. acuta*-type) at the same time in addition to *T. amphioxeia*-type plastid in a single cell. Interestingly, SfaNI digestion of the products amplified on psbA gene from 10 *Dinophysis* cells produced a different RFLP pattern: in addition to *T. amphioxeia*-type and sometimes *T. acuta*-type plastid, undigested fragments occurred.

PHYLOGENY OF EUGLENOID AND GREEN ALGAL CHLOROPLASTS USING SSU RDNA AND TRNA'S

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The SSU rDNA and tRNA genes from euglenoid and green algal chloroplasts were used to create a phylogeny which was used to infer the endosymbiotic origin of the euglenoid chloroplast. A 12 gene dataset was aligned and a phylogeny was inferred using Bayesian methods. The tree placed *Pyramimonas parkeae* at the base of the euglenoid lineage suggesting that *Pyramimonas* was most closely related to an ancestral chloroplast donor. While the basal position of *Pyramimonas* was well supported, relationships among several euglenoid species were not resolved. *Euglena viridis* was not included in the same clade as the other two Euglena species, causing the genus to be polyphyletic. The position of *Strombomonas acuminata, Euglenaria anabaena,* and *Euglena viridis* differs from previous trees based on nuclear encoded genes. These results may suggest that there was insufficient phylogenetic signal in the dataset and more taxa and/or genes may be needed to resolve the relationships among these taxa. Alternatively, the results may indicate that the genes selected may not be under strong selection pressure and may be evolving at differing rates in the various lineages.

EVOLUTIONARY CHANGES IN THE EUGLENOID CHLOROPLAST

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The chloroplast sequences of four photosynthetic euglenoids including *Colacium vesiculosum*, *Euglenaria anabaena*, *Eutreptia viridis*, and *Strombomonas acuminata* are near completion. This has been achieved with Roche 454 sequencing and the manual assembly of contigs. These chloroplast genomes offer a snapshot into the evolution of this highly variable group of organisms, which acquired their chloroplasts through secondary endosymbiosis. Through the use of synteny mapping, large gene rearrangements have been observed ranging from single gene movements to the flip-flopping of large blocks of genes. Comparisons between taxa have confirmed the presence of key chloroplast genes suggesting a high level of gene conservation within the lineage. Few genes have been identified as absent, but bring to light the need to identify methods of gene loss and/or transfer within the euglenoids. Further comparisons with the chloroplast genome of *Euglena gracilis* and close green algal taxa have provided insight into euglenoid chloroplast evolution, and in doing so has indentified the putative green algal chloroplast donor as *Pyramimonas parkeae.* The sequencing of additional chloroplast genomes will undoubtedly refine the evolutionary history of the euglenoid chloroplast lineage.

A COMPARISON OF RIBOSOMAL AND PROTEIN BAYESIAN PHYLOGENIES WITHIN THE PHOTOSYNTHETIC EUGLENOIDS

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The purpose of this study was to incorporate protein coding genes into euglenoid phylogenies and compare the results with those of ribosomal genes. The psbO gene, a nuclear encoded plastid targeting gene involved in oxygen evolution, and the HSP90 gene, a nuclear encoded heat shock gene that codes for a molecular chaperone, were sequenced for 69 photosynthetic euglenoid taxa. Nuclear SSU, LSU, and chloroplast 16S ribosomal sequences were also obtained for the euglenoid taxa from previous ribosomal studies (Triemer et al. 2006). The phylogenies inferred from the ribosomal genes resolved most relationships among the genera within the euglenoids as evidenced by very high Bayesian posterior probabilities. In contrast, the phylogenies inferred from psbO and HSP90 resulted in trees that resolved very few clades but resolved many deep relationships between species within the same genera. These results suggest that ribosomal analyses provide very robust phylogenies at the generic level whereas protein analyses were more useful at the species level. Furthermore, when the ribosomal and protein sequences were combined into a single phylogeny, the results suggested that a combined analysis is preferred over either alone.

MOLECULAR AND MORPHOLOGICAL CHARACTERIZATION OF TINTINNID CILIATES

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Despite recent growth in genetic and genomic approaches to understanding the diversity of protists, the relationship between DNA sequences and the classical criteria for species identification remains controversial. In this context, tintinnid ciliates represent a challenging case, as taxonomy is based mainly on the morphology of the lorica, a structure with questionable diagnostic value due to its plasticity. The aim of this study is to compare the molecular and morphological variability within and between tintinnid species from coastal waters of the North and South Atlantic. Individual organisms were photographed to document morphology and subjected to sequencing of the nuclear small subunit rDNA. Species in the genera *Tintinnopsis, Tintinnidium, Helicostomella, Eutintinnus* and *Favella* were analyzed. We found species showing morphological overlap but divergent sequences as well as species with different morphology but identical sequences. Species within the genus *Tintinnopsis* were in some cases more distant from species in their own genus than they were to those in *Helicostomella* or other genera previously sequenced, regardless of the presence of an agglomerated or hyaline lorica. These results are expected to contribute to accurate systematic and phylogenetic schemes for tintinnids.

PROROCENTRUM MEXICANUM VS. *PROROCENTRUM RHATHYMUM*: COMPARATIVE TAXONOMY, ECOLOGY AND TOXICOLOGY

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Considerable confusion exists in the literature concerning the ecology and potential toxicity of the important *Prorocentrum* species *P. mexicanum* and *P. rhathymum. Prorocentrum mexicanum* was first described by Osorio-Tafall in 1942 and *P. rhathymum* by Loeblich et al. in 1979. Because of the similarities between the original line drawings of *P. mexicanum* and those of *P. rhathymum*, both species were deemed to be synonymous in 1982. In 2003, Cortés-Altamirano and Sierra-Beltrán presented convincing evidence, based on the original written description of *P. mexicanum*, that the two species were distinct. Thus, over 20 years of literature concerning these species is confused. To address this confusion, we first established single cell isolates of each species which allowed a more comprehensive morphological and molecular characterization than previously available. The resulting detailed SEMs and rDNA phylogenies highlighting the differences between these two species will be presented. In addition, an extensive literature survey listing those studies where unambiguous species identification was possible will be provided along with a more accurate summary of the ecology, distribution, and toxicology of each species.

LIMU FROM THE LOI: MACROALGAE ASSOCIATED WITH TARO FIELDS IN THE HAWAIIAN ISLANDS

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Taro (*Colocasia esculenta*) is a culturally significant food crop in Hawaii. Freshwater macroalgae (*limu*) have been a recognized component of taro cultivation systems in the Hawaiian Islands for hundreds of years, and several Hawaiian names are known to describe these taxa. Taro fields, or *lo'i*, often contain both fishes and macroalgae as part of the nutrient cycling system of the fields. We collected macroalgae from taro fields on the five largest Main Hawaiian Islands as part of our ongoing Freshwater Algae Biodiversity Survey of Hawaii because these fields represent a unique freshwater habitat in the Hawaiian Archipelago. Ninety-six collections were made, resulting in 170 macroalgal identifications spanning 34 genera; these identifications were compared to historical literature records dating back to 1901. DNA sequence comparisons of multiple markers (UPA, *rbcL*, 18S, COI, *tufA*) were used to identify genotypes associated with taro fields versus other freshwater habitats. Some taxa were found to be associated with multiple habitats (e.g. *Compsopogon caeruleus, Cloniophora spicata*), while others were exclusively collected from taro fields (e.g. *Hydrodictyon reticulatum*, some *Spirogyra* spp.).

THE BENTHIC MARINE ALGAE OF THE TROPICAL AND SUBTROPICAL WESTERN ATLANTIC: CHANGES IN OUR UNDERSTANDING IN THE LAST HALF CENTURY

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The volume ''Marine algae of the eastern tropical and subtropical coasts of the Americas'' produced by W. R. Taylor in 1960 was a comprehensive treatment of the benthic seaweeds of this region and remains a useful compendium for the breadth of its coverage, its detailed descriptions, and excellent artwork. But in the 50 years since that floristic treatment, numerous regional treatments have appeared, many new taxa described, and many new records have been published. Wynne has produced several checklists over the years (1968, 1998, 2005) in efforts to gather the pertinent literature from this broad region from Cape Hatteras, North Carolina, in the north to southern Brazil in the south. The recently published third revision (Wynne 2011) gives us the opportunity to see the dramatic changes in the schemes of classification and in the treatment of genera of green, brown, and red algae from the new information provided by the flood of phylogenetic data. These changes will be presented with some general and specific examples.

ARRANGEMENT OF THE PERICENTRAL CELLS IN TRIBES OF THE RHODOMELACEAE (CERAMIALES, RHODOPHYTA) THAT EXHIBIT PRIMARY DORSIVENTRALITY

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Earlier, Hommersand distinguished between primary dorsiventrality in the Rhodomelaceae in which symmetry is established at the apex at the time the pericentral cells are initiated and secondary dorsiventrality that results from the displacement of side branches out of the plane of radial or bilateral symmetry. Pericentral cells are cut off in an alternating sequence in Rhodomelaceae with the 2nd next to the 1st, the 3rd next 1st on the other side, the 4th next to the 2nd, etc. Primary dorsiventrality is established in the tribes Bostrycheae, Herposiphoneae, Polyzonieae and Pleurostichideae with a shift in the direction of pericentral cell formation taking place every time a polysiphononous exogenous branch is formed. In the Amansieae dorsiventrality is consistently related to the pattern of trichoblast (monosiphonous hair) formation on the dorsal side with the trichoblasts situated between the 1st and the 2nd pericentral cells, and polysiphonous branches are formed endogenously from the ventral side. Branching is dorsiventral in the usual way in the tribe Streblocladieae; however, this group falls within the radially branched tribe Polysiphoneae in molecular analyses.

GRATOL – ASSEMBLING THE GREEN ALGAL TREE OF LIFE

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Green algae are an understudied, yet diverse group of eukaryotes with critical ecological roles in terrestrial and aquatic habitats, with diverse life styles ranging from autotrophic to symbiotic (including parasitic), and of particular prominence because embryophytes inherited their basic genetic toolkit from green algal ancestors. The overall goal of GrAToL is a new classification for all major branches of green algae. The project includes over 25 scientists and trainees contributing taxonomic expertise and extensive knowledge of data collection and analysis. We are using a hybrid approach of PCR-based multigene sequencing, morphology and next-generation sequencing methods. The team will collect data from 10 genes for 425 taxa, sampled from the nucleus, mitochondria, and plastid genomes for phylogenetic analysis. Next-generation sequencing of 16 taxa will generate data to study deep phylogenetic relationships in the green algae, identify new target genes for detailed analysis of particular groups, and examine organellar genome evolution. A public web site includes a library of images, phylogenetic trees, and other resources. We will disseminate new phylogenetic analysis methods through free, open-source software.

COMPARATIVE ULTRASTRUCTURE OF THE SYMBIONTIDA (EUGLENOZOA)

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Postgaardi is a euglenozoan that lives in low oxygen environments and is covered with episymbiotic bacteria. The Symbiontida is a distinct euglenozoan subgroup that initially included only two genera, namely *Calkinsia* and *Bihospites.* Both of these genera possess rod-shaped episymbiotic bacteria like that found on *Postgaardi.* The ultrastructure of *Postgaardi* was described more than a decade ago and no further studies have been carried out since. In the absence of molecular phylogenetic data, we re-examined the ultrastructure of *Postgaardi* in order to help elucidate the phylogenetic position of this species within the Euglenozoa.We demonstrated that *Postgaardi* has a feeding apparatus consisting of five fibrous ''fingers''. This distinctive feeding complex is also found in *Calkinsia*, but nowhere else within the Euglenozoa. The pellicle and complex feeding apparatus of *Bihospites* suggests that the Symbiontida is nested within phagotrophic euglenids. Therefore, this work enabled us to establish evidence that *Calkinsia* and *Postgaardi* are closely related to each other, and their feeding apparatus was significantly reduced in their most recent common ancestor.

POPULATION STRUCTURE AND COLONIZATION HISTORY OF A COSMOPOLITAN BROWN ALGA, *COLPOMENIA PEREGRINA*

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We assessed the genetic structure of populations of a widely distributed brown alga *Colpomenia peregrina*, and investigated the effects of marine barriers to gene flow and historical processes. Several potential genetic breaks were considered, which would separate by intercontinental distances between Asia and Europe or Oceania or America. We analyzed mitochondrial cox3 and atp6 gene sequences from 240 individuals from 28 locations: 14 in Asia, five Europe, four Oceania, and five America. Haplotype diversity was high (H = 0.866) in Asia, while low (H = 0.331) in other regions. The lowest genetic diversity was found in the European populations. Haplotype network analyses revealed the global colonization history of *C. peregrina*, providing evidence of multiple invasions to Europe, secondary invasions to Atlantic North America. The distribution of haplotypes, the pairwise FST values and the results of exact tests, AMOVA, and mismatch distribution revealed: (1) a significant genetic break between populations in Europe and other locations, (2) weak differentiation of Asian populations from the other populations, (3) strong signals of recent expansion within Asian and Oceania populations, respectively. The historical population expansion of the species predated probably the last glacial maximum (LGM). Historical events, together with the current oceanographic patterns, were proposed as the main factors that determine the population structure and genetic signature of *C. peregrina.*

SYSTEMATICS OF THE GENUS *CRYPTOGLENA* (EUGLENACEAE) BASED ON MOLECULAR AND MORPHOLOGICAL CHARACTERS

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The genus *Cryptoglena* of photosynthetic euglenoids has differentiated from other euglenoid genera by a longitudinal sulcus, one chloroplast, two paramylons positioned between the chloroplast and pellicle, and lack of metaboly, and is consisted of only two species defined by molecular signatures up to date. To understand genetic diversity and taxonomy of two *Cryptoglena* species, we analyzed molecular and morphological data from 26 strains. A combined data set of nuclear SSU and LSU and plastid SSU and LSU rDNA was analyzed using Bayesian, maximum likelihood (ML), maximum parsimony (MP), and distance (NJ) methods. Although morphological data of all strains showed no significant species specific pattern, molecular data supported that the genus *Cryptoglena* was divided into six clades: *C. skujae* clade, *Cryptoglena* sp. 1 clade, *C. pigra* clade, *Cryptoglena* sp. 2 clade, *Cryptoglena* sp. 3 clade, and *Cryptoglena* sp. 4 clade. Previously known species, *C. skujae* and *C. pigra*, and additional four clades were well recovered with strong supportive as monophyletic lineages. Therefore, we propose four new species based on specific molecular signatures and gene divergence of the nr SSU rDNA sequences.

THE FLAGELLAR APPARATUS OF *RHODOMONAS SALINA*

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Rhodomonas salina Hill & Wetherbee is a photosynthetic marine flagellate that is known to have typical characteristics of the cryptomonads. The ultrastructure of *R. salina* was examined with particular attention to the flagellar apparatus and compared with those of other cryptomonads reported previously. The major components of the flagellar apparatus of *R. salina* CCMP1419 were keeled rhizostyle(Rhs), six-stranded microtubular root(6r), striated root(SR), SR associated microtubular root(SRm), and anchoring fiber(AF). The Rhs originated near the proximal end of the dorsal basal body, passed through the left side of gullet and finally dissociated at the posterior of the cell. In cross section, the Rhs composed of a band of four microtubules. At the level of nucleus, only three out of four microtubules had wing structure. The 6r overlapped with the Rhs at the basal bodies, picked up three additional microtubules and extended toward dorsal anterior lobe of the cell. The SR originated between two basal bodies and laterally extended to the right side of cell. The SRm was associated with the SR and composed of three microtubules. The AF connected the ventral basal body to the SRm. The overall configuration of the flagellar apparatus in *R. salina* was similar to those of Cryptomonas paramecium, *C. ovata, Cryptomonas* sp. Φ and *θ*. The ultrastructural differences of the flagellar apparatus among cryptomonad species will be discussed.

THREE NEW *MALLOMONAS* SPECIES (SYNUROPHYCEAE) FROM KOREA AS INFERRED FROM MOLECULAR AND MORPHOLOGICAL DATA

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Mallomonas is a unicellular genus characterized by having silica scales and bristles. Classification of species is based mainly on ultrastructural characteristics of the siliceous cell covering. In this study, three new *Mallomonas* species from Korea, *Mallomonas* sp. nov. 1, 2 and 3, were described using ultrastructure and molecular data. All species were members of the section Planae due to lack of a V-rib and dome of the scales. *M.* sp. nov. 1 cells were ellipsoidal to ovoid, 18–22 × 8–13 µm in size. The bristle was terminated in expanded tip and the scale shield was marked with thickly spaced secondary meshwork and pore except posterior region. *M.* sp. nov. 2 cells were ellipsoidal to ovoid, 28–36 µm × 7–15 µm in size. The bristle was terminated in bifurcate tip and the scales shield was marked with small papillae except posterior region. *M.* sp. nov. 3 cells were rounded oval, 12–18 × 7–12 µm in size. The bristle was terminated in several parted tip and the scales shield was marked with spaced secondary meshwork except posterior region. The molecular phylogenetic position of *Mallomonas* sp. nov. 1, 2 and 3 were inferred using nuclear SSU and LSU rDNA and plastid rbcL sequences. In analyses, newly described *Mallomonas* species were strongly supported as members of the section Planae. Members of the new species were subdivided into two subclades. *Mallomonas* sp. nov. 1 was closely related to *M. matvienkoae. Mallomonas* sp. nov. 2 was closely related to *Mallomonas* sp. nov. 3.

PHYLOGENETIC INFERENCE OF POSITIVE SELECTION ON A DUPLICATED PHOTOSYNTHESIS GENE IN THE DIATOMS

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The protochlorophyllide oxidoreductase (POR) enzyme catalyzes a light-dependent step in the chlorophyll synthesis pathway. In diatoms, a duplication event has occurred in the gene (por) encoding this enzyme. Two unique por genes are present in each of the three sequenced diatom nuclear genomes *Fragilariopsis cylindrus*, *Phaeodactylum tricornutum*, and *Thalassiosira pseudonana*, whereas only one por gene is present in the nuclear genomes of the Pelagophyte *Aureococcus anophagefferens* and Phaeophycean *Ectocarpus siliculosus*. The diatom por genes form two clades in a por gene phylogeny, demonstrating a single duplication event. A maximum likelihood method for detecting positive selection on particular lineages of a phylogenetic tree identified positive selection on a diatom por lineage. Approximately six amino acids (of 433) encoded in this por gene were identified as having undergone positive selection (depending on model parameters). Notably, the other diatom por gene is predicted to encode a ~150 amino acid C-terminal extension when compared to other known por genes.

CONCORDANT ECOLOGICAL AND GENETIC DATA RESHAPE OUR UNDERSTANDING OF DINOFLAGELLATE SPECIES BOUNDARIES

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Current understanding of *Symbiodinium* diversity is based primarily on analysis of rDNA spacer regions (ITS1 and ITS2). While hundreds of ribotypes exhibiting distinct ecological and biogeographical distributions are documented, species assignments remain a subject of controversy. Despite possessing very similar ITS2 sequences, *S. glynni* associates with reef corals in the genus *Pocillopora* sp. while *S. trenchi* associates with a wide variety of cnidarian host taxa, and therefore occupy separate niches. The validity of these informal putative ''species'' was tested by multi-locus genotyping. Using 13 microsatellite loci, we demonstrate that these taxa do not share a common gene pool in sympatry. Indeed, greater genetic differentiation exists between co-occurring populations of *S. trenchi* and *S. glynni* than between widely distributed populations of each ''species''. *Symbiodinium trenchi* and *S. glynni* are further distinguished by a genome duplication event and a non-synonymous mutation probably affecting the function of the chloroplast psbA gene. These observations demonstrate the utility of using multiple lines of evidence for delimiting species of *Symbiodinium* and indicate the need for major changes in rules governing the taxonomy of eukaryotic microbes.

SYSTEMATICS OF HALARACHNION (FURCELLARIACEAE, RHODOPHYTA), INCLUDING A NEW SPECIES FROM OFFSHORE LOUISIANA, NW GULF OF MEXICO

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Halarachnion Kützing is a newly reported genus for the Gulf of Mexico where it is represented by an undescribed species growing attached to rhodoliths at 52–73 m depth in the northwestern Gulf, offshore Louisiana. Aspects of the vegetative and female reproductive morphology of the Gulf species are documented, and compared to those of *Halarachnion latissimun* from Japan. *Halarachnion* sp. nov. is a monoecious, presumably heteromorphic species, as inferred from the absence of tetrasporophytic specimens in our collections. *H. latissimum*, in contrast, is a dioecious species that is isomorphic. Comparative chloroplast-encoded rbcL sequence analysis indicates that isolates from the Gulf of Mexico are more closely related to *H. ligulatum* from the Atlantic Ocean than to *H. latissimum* from Japan. This is the first report of the Furcellariaceae, a family in the Solieriaceae-complex, for the Gulf of Mexico.

THE CHLOROPLAST GENOME OF *EUGLENA VIRIDIS*

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It has been believed for some time that the origin of the euglenoid chloroplast was through a secondary endosybiotic event wherein a phagotrophic euglenoid engulfed a green algal cell, kept the green algal chloroplast, and utilized that chloroplast for its' own benefit. To date, there are only two published euglenoid chloroplast genomes: those of *Euglena gracilis* and the colorless *Euglena (Astasia) longa*, which has secondarily lost the ability to photosynthesize. While these genomes were important first steps to understanding euglenoid chloroplast origins and evolution, much more work needs to be done to explore these processes. In an effort to begin investigating these ideas, we have sequenced the chloroplast genome of the

photosynthetic *Euglena viridis* using ‘‘next-generation’’ sequencing technology. After gene annotation, the genome was compared to the previously published euglenoid chloroplast genomes and analyzed for synteny, gene insertions and deletions, and sequence similarities. Based on our draft chloroplast genome, it is clear that most of the genes found in *E. gracilis* are also present in *E. viridis*. However, we have found examples of gene rearrangements where two genes have not only reversed their order, but have changed strands, and other genes that are missing entirely from the chloroplast genome.

A NEW SPECIES OF *CALOGLOSSA* (DELESSERIACEAE, RHODOPHYTA) FROM THE COASTLINE OF THE WESTERN ATLANTIC

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The red alga genus *Caloglossa* (Harvey) G. Martens is represented by several species along the coastline of the Western Atlantic. Even though the genus has already been the focus many taxonomic studies, its diversity is not yet fully understood. Comparative chloroplast-encoded rbcL and nuclear 26S LSU rDNA sequence analyses, combined with morphological observations, reveal a new species for the coastline of the Western Atlantic. This new species collected from intertidal localities in Florida USA, Mexico, and Brazil is characterized by thalli of ovate blades, with endogenous branching, and strong constrictions at the thallus nodes. The proposed new species forms a monophyletic group with other species whose only mode of secondary branching is also endogenous. The taxonomic importance of rhizoid morphology, degree of constriction at the thallus nodes, secondary branching pattern, blade morphology, blade width, number of cell rows cut off from the first axial cell of the main axis, and presence of an adaxial cell row derived from the first axial cell from lateral axis is illustrated and discussed.

LIGHT AND SCANNING ELECTRON MICROSCOPE OBSERVATIONS OF THE DIATOM GENERA *LICMOPHORA* AGARDH AND *PODOCYSTIS* BAILEY IN THE NORTHEASTERN GULF OF MEXICO

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We are currently re-assessing the state of the diatom communities in the northeastern Gulf of Mexico in response to the recent oil spill. Included among the sea grass epiphytes of the local bays is a diverse assemblage of species belonging to the genera *Licmophora* and *Podocystis*. Included among these is an interesting form with affinities to both genera. The new form occurs as solitary or paired cells, with numerous discoid plastids. Frustules are heterovalvate, with one or two rimoportulae per valve. Valves are heteropolar with a narrow base, broadened center, and rounded head. Rimoportulae, always present at the head pole, are simple in structure, with orientation ranging from parallel to perpendicular to the apical axis. Rimoportulae at the base-pole, when present, are larger, fan-shaped, oriented perpendicular to the apical axis. Striae biseriate, separated by thickened costae. The base-pole contains a multiscissura. The presence of a multiscissura suggests affinity with *Licmophora*, while the presence of biseriate striae and thickened costae and the morphology of the rimoportulae suggest *Podocystis*. This combination of features precludes a definitive placement at this time.

ANALYSIS OF TRANSCRIPTOMES FROM THREE MICROBIAL EUKARYOTES

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Resolving relationships across the eukaryotic tree of life is a major goal in modern biology. While the amount of molecular data from microbial eukaryotes has grown tremendously, the difficulty of generating genome-scale data from the number of taxa necessary to resolve deep relationships continues to make this goal an elusive one. Eukaryotic transcriptomes are smaller and simpler than their genomes, so RNA-Seq is a more efficient way to increase the availability of sequence data than whole genome sequencing or traditional methods. Although producing data from the hundreds of lineages needed to span the tree of life is still a daunting task, we believe that increased data from targeted and thoughtfully chosen taxa can greatly enhance studies of the comparative biology of eukaryotes. We present RNA-Seq data from *Chilodonella uncinata*, a ciliate; *Corallomyxa tenera*, a taxon in the Rhizaria; and Subulatomonas tetraspora, a microaerophilic amoeboflagellate that is a member of a group that falls outside the major clades of eukaryotes. Our analyses yield insights into the biology of these organisms, and will allow phylogenomic placement of these lineages.

A CRITICAL EXAMINATION OF THE GENUS *CHRYSYMENIA* (RHODYMENIALES, RHODOPHYTA), WITH A SPECIAL EMPHASIS ON TAXA FROM THE GULF OF MEXICO

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Chrysymenia J. Agardh 1842, a genus within the Rhodymeniaceae Harvey 1849, is currently reported as comprised of 19 species with a primarily warm-temperate to tropical distribution. It has been historically defined by a hollow thallus lacking both diaphragms and internal rhizoids, and with a solid portion limited to the stipe. Whereas Cryptarachne (Harvey) Kylin 1931, since merged with *Chrysymenia*, was characterized by the presence of internal rhizoids, *Chrysymenia* now includes species that may or may not contain such rhizoids. The present study investigates 13 taxa placed in both genera to establish more resolved generic concepts within a larger context of Rhodymeniaceae on the basis of chloroplast encoded rbcL and nuclear-encoded LSU rDNA gene sequence analysis, and morphological observations. Worldwide samples investigated include the generitype, *Chrysymenia ventricosa* (J. V. Lamouroux) J. Agardh 1842. The majority of the collections originated from the Gulf of Mexico, including three, well-resolved, new species of *Chrysymenia*.

EFFECTS OF THE ANTHROPOGENIC APRIL 2010 BP DEEPWATER HORIZON CRUDE OIL SPILL ON SEAWEED DIVERSITY IN THE NW GULF OF MEXICO

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Two 5-day ship-based dredging expeditions centered on previously studied hard banks throughout the Gulf to assess the impacts of the Deepwater Horizon oil spill on the diversity, vitality, and distribution of offshore seaweeds living principally at 45–90 m depth. Results analyzed from the first 5-day leg of the expedition offshore Louisiana, Mississippi and Alabama (December 2010) indicate that macroalgal diversity of the previously documented assemblage appeared limited in all dredged sites. The six species of seaweeds dredged typically have a zone of meristematic surface cells or are embedded in a gelatinous matrix. Strong petroleum odors were pervasive when the dredge was retrieved from ~55 m depth at Ewing Bank 2 (Fish Haven) far west of the Deepwater Horizon Explosion site; samples included individual carbonate nodules covered by light crude oil. The material is awaiting final HC fingerprinting analysis. In the second leg of the RAPID expedition, April 2011, seaweed diversity had increased to ~20 species. Additional seasonal dredging trips are urgently needed to fully assess whether the marine flora shows evidence of recovering to pre-event summer levels.

STUDIES ON THE DIVERSITY OF SUBAERIAL ALGAE IN SOUTHERN GEORGIA: POTENTIAL CRYPTIC SPECIATION IN *COELASTRELLA* (*CHLOROPHYCEAE, CHLOROPHYTA*)

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We have isolated a number of strains of subaerial algae from southern Georgia as part of a larger effort aimed at (1) determining the diversity of subaerial algae in the region and (2) elucidating the mechanisms enabling them to thrive with limited access to liquid water. Morphological investigations of two of these strains placed them within the genus *Coelastrella* (Chlorophyta, Scenedesmaceae). Analysis of the nuclear 18S rRNA gene placed one of the strains in close proximity to *Coelastrella saipanensis* and *C. vacuolata*. Morphological data, including the presence, number, and structure of meridional ribs in the cell walls, suggests that these taxa are distinct and should be considered a separate species. Analysis of the 18S rRNA gene placed a second strain in close proximity to *Coelastrella multistriata*. Although the two entities differ significantly in their 18S rRNA genes, we have not identified specific morphological differences, suggesting the possibility of cryptic speciation.

MOLECULAR PHYLOGEOGRAPHY OF *GELIDIUM ELEGANS* (GELIDIALES, RHODOPHYTA) IN THE NORTHWEST PACIFIC OCEAN

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Gelidium elegans is morphologically a variable species that occurs commonly in the northwest Pacific Ocean. In order to assess genetic structure of the species in the range of distribution, we analysed mitochondrial cox1 sequences from 272 individuals collected in fifteen locations in Korea, China, and Japan. Haplotype diversity was high in central Japan (H = 0.780) and western Korea (H = 0.685), while the nucleotide diversity was relatively low in most of locations. A total of 34 haplotype were recovered, including 28 (87.5%) private's. Haplotype C4 was widely distributed from China via Korea to northern Japan, and haplotype C1 was the most abundant in Korea and southern Japan. The distribution of cox1 haplotypes, pairwise FST values, results of neutrality tests, AMOVA, and mismatch distribution revealed (1) a significant genetic break between central Japan and the other locations, (2) a gene flow within the range except central Japan, and (3) several potential demographic expansions. Historical events and the current oceanographic patterns were discussed as the main factors that determine the population structure of G. elegans.

NEW INSIGHTS ON THE ORIGIN OF *PORPHYRA* SPECIES (BANGIALES, RHODOPHYTA) FROM BRAZIL

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Traditionally, only the genera *Bangia* and *Porphyra* are accepted in the order Bangiales (Bangiophyceae, Rhodophyta). However, a novel classification is being proposed based on robust phylogenetic analyses, including specimens of Bangiales collected worldwide. In this classification, 15 genera will be recognized, with eight representing the foliose specimens and seven representing the filamentous one.

One of the foliose genus comprises the species that occur on the Brazilian coast, for now still treated as *Porphyra*, which group together with about 75 species including the edible species from Japan commercialized as ''nori''. Recent studies on the Brazilian *Porphyra* flora, based on molecular tools, have indicated three new occurrences on our shore: *Porphyra* sp. Piaui, *Porphyra* sp. 77 and *P. suborbiculata*. In this study, we investigated the phylogenetic relationships of this three species with the other Brazilian species. The two first were a match to the Asian species *P. vietnamensis* and *P. tanegashimensis* respectively, and were resolved within the same clade of *P. suborbiculata* and the Brazilian *P. acanthophora*. Based on these results, we propose new hypothesis on the origin of Brazilian *Porphyra* species.

DIVERSITY AND DISTRIBUTION OF EPIPHYTIC SUBAERIAL ALGAE IN A PANAMANIAN FOREST

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An environmental sequencing approach was used to investigate the biodiversity of the epiphytic algal community in a Panamanian tropical rainforest. Cloning techniques were carried out on amplicons derived from the 23S rDNA of the plastid genome. A total of 186 sequences were generated, with an average length of 360 bp. The maximum numbers of algal phylotypes was 173 at 99% similarity. The maximum numbers of phylotypes at 98% and 97% were 125 and 88 respectively. Through phylogenetic analysis, the phylotypes were inferred into two major algal lineages (cyanobacteria and chlorophytan algae). The cyanobacteria had the highest number of phylotypes followed by the chlorophytes including the order Trentepohliales. The majority of inferred cyanobacterial sequences consisted of species with thick mucilaginous sheaths while chlorophyte sequences were mainly inferred from the trebouxiophyceae (phycobionts). By applying environmental sequencing techniques the observed algal diversity of subaerial epiphytes yielded higher taxa than traditional morphology-based approaches.

SPHAEROSPERMOPSIS TORQUES-REGINAE (CYANOBACTERIA, NOSTOCALES) COMB. NOV. FROM SOUTH AMERICAN WATER BLOOMS

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Sphaerospermopsis torques-reginae (Komárek) comb.-nov. was originally described as An*abaena torques-reginae* Komárek from planktonic populations of Cuban eutrophic environments, characterized by twisted trichomes with spherical akinetes adjacent to heterocytes. Recently, all planktonic *Anabaena* morphospecies were transferred into the genus *Dolichospermum*. However, through a polyphasic characterization of strains of *Anabaena reniformis* Lemmermann and *Aphanizomenon* (=*Anabaena*) *aphanizomenoides* (Forti) Horecká et Komárek, these species were reclassified into *Sphaerospermopsis*. This study's main objective was to morphologically and molecularly characterize cyanobacterial populations identified as *D. torques-reginae* from different aquatic ecosystems in South America. The 16SrRNA gene of two *D. torques-reginae* strains (ITEP024 and ITEP026) was used for phylogenetic analysis. The morphological and phylogenetic analyses demonstrated the affiliation of our populations within the genus *Sphaerospermopsis*. Furthermore, geographic distribution, ecology, and toxicity of the species are discussed. *Sphaerospermopsis torques-reginae* was observed in Brazil, Argentina, and Colombia, suggesting a wide distribution in South America. It normally occurred in dense freshwater blooms, although also found in water with low salinity. *Sphaerospermopsis torques-reginae* toxic blooms, identified as *Anabaena spiroides* Klebahn, producing anatoxin-a(s) have been registered in northeastern Brazil.

ENHANCEMENT OF THE HIGH TEMPERATURE STRESS TOLERANCE BY THE *PORPHYRA SERIATA* HSP70 IN CHLAMYDOMONASE

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Temperature is one of the major environmental factors effects on the distribution and growth rate of the intertidal organism including red algae. To identify genes involved in the high temperature tolerance of *Porphyra*, we generate 3,988 ESTs from gametephyte thallus of *P. seriata* under normal growth condition and high temperature condition. We identify the transcripts highly abundant in heat treated thallus by comparison of the ESTs from two difference tallus and called them as HTR (high temperature response) cDNA. Among HTRs, the HTR5 encode a HSP70. Through analyses of the P. seriata ESTs, we identify and characterize the nine HSP cDNAs, which encode various molecular weight HSPs from 95.6 kDa to 25.8 kDa. RT-PCR results show that the transcript of

the HTR5_HSP70 gene was increase by high temperature. To check the biological function, we introduce the HTR5_HSP70 gene into *Chlamydomonas reinhardtii.* Transformed *Chlamydomonas* with over expressed HTR5_HSP70 show higher survival and growth rate than those of the wild-type under high temperature treatment condition.

TRANSCRIPTOME SEQUENCING AND COMPARATIVE ANALYSIS OF THE *PORPHYRA TENERA* UNDER ABIOTIC STRESS CONDITION

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A marine red alga *Porphyra tenera* grow in the intertidal zone which undergoes dynamic environmental changes including temperature, desiccation, osmotic shock, and light intensity. Therefore, intertidal organisms such as *Porphyra* that grow on intertidal rocks have developed a variety of strategies and mechanisms to survive through those environmental stresses. In an effort to identify the genes involved in the high-temperature tolerance of *Porphyra*, we generated a total of 1,280,745 high quality EST sequences using Roche-454 massive parallel pyrosequencing technology. Among the total read, 308,856 were from normal growth condition and 313,710 from high temperature condition, 390,322 from dry condition and 267,857 from freezing temperature condition. These EST sequences were clustered and assembled into 8,218 unigenes. Comparison of the ESTs from tallus under different stress condition enable us to identify the transcripts up- or down regulated by high temperature, freezing or desiccation stress. These ESTs will provide valuable information to further understand molecular mechanisms of the abiotic stress tolerance.

DECIPHERING RNA PROCESSING PATHWAYS IN *AMPHIDINIUM CARTERAE* PLASTIDS

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The plastid genomes of peridinin-containing dinoflagellates have a highly unusual organisation, with genes encoded on multiple small minicircles. The pathways by which minicircle-derived transcripts are generated and processed are poorly understood, but show a number of idiosyncratic features including transcription via a rolling circle mechanism, and 3’ terminal uridylylation of transcripts. We present the outcomes of a series of RT-PCR studies designed to detect transcriptional intermediates indicative of transcript processing events in the model dinoflagellate *Amphidinium carterae.* We demonstrate that transcription occurs in *A. carterae* via a rolling circle mechanism, and that polycistronic transcripts are apparently processed to mature monomers following polyuridylylation. We present evidence for the production of multiple alternate monocistronic transcripts from *A. carterae* minicircles, including several regions containing ORFs previously not known to be expressed. Finally, we identify the presence of both polyuridylylated and nonpolyuridylylated transcripts from circularised *A. carterae* RNA. Quantitative analysis of circularised psbA sequences suggests that polyuridylylation acts to protect the 3’ ends of transcripts from degradation, and to define specific 5’ ends in transcript processing pathways.

HOW DOES *PAULINELLA CHROMATOPHORA* (EUGLYPHIDA, CERCOZOA) BUILD A DAUGHTER’S SHELL?

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Paulinella chromatophora is a euglyphid testate amoeba and has attracted much attention in recent years to have a blue-green photosynthetic organelle that has derived from a cyanobacterium via an independent primary endosymbiosis. However, the basic information, such as proliferation manner, has not been understood well. Most euglyphid species, including *P. chromatophora*, have a signature ovoid shell composed of siliceous scales. Unlike other siliceous shell forming protists, *P. chromatophora* forms a complete empty shell outside of the cell before a daughter cell goes into it during cell division. It is a big mystery how a single cell does it. By detailed time lapse video observations, we found that, the cells secreted the scales from an aperture of own shell, piled up the scales with a thick pseudopodium and a daughter cell moved into a new shell. Although small scales near the aperture was made first in the mother cell, large scales around the middle of the shell were secreted first. It is also suggested that the scales are bonded each other with a cement-like material which probably contains N-acetyl-galactosamine-alpha 1,3-Galactose.

LET YOUR IMAGES OF PROTISTA POPULATE THE CELL: AN IMAGE LIBRARY

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We need your images of Protista so they can get their deserved respect. Let me help you populate the library with your high resolution, high quality images as I am directly involved with the development of this cell biology resource. The ASCB Cell Image Library provides research quality images to and for Researchers, Educators, Students, and the Lay Public by allowing for the viewing and download of data. The worldwide site is expected to allow for collaboration among researchers who might not know each other. In this way, the synergy of these images is expected to archive objectives like GenBank. Since it's in it's infancy, your research and research program cannot help but to benefit from it's exposure. My involvement in this project is, in part, due to my desire to more broadly share and recognize the contributions of protistan research to our understanding of basic cell biology. Please consider sharing your images through me, and I will try to give your work the best exposure possible. To look at the library go to http://www.cellimagelibrary.org/.

TRACING CARBON FLOW INTO NEUTRAL LIPIDS IN THE MARINE HAPTOPHYTE *EMILIANIA HUXLEYI*: CLUES TO BIOSYNTHETIC PATHWAYS

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E. huxleyi, a globally important coccolithophorid, synthesizes an unusual set of polyunsaturated long-chain alkenones, alkenoates, and alkenes (PULCA) as its neutral lipids, which are stored in lipid bodies similar to the triacylglycerols produced by most other algae. These C37–39 hydrocarbons with 2–4 trans-alkene bonds occurring at 7-carbon intervals are structurally attractive as a possible biofuel. However, little is known about their biosynthesis. Here, I monitored carbon flow into PULCA stores using radiotracers. Stationary phase cultures of strains CCMP 1516 and 371 were pulse and pulse-chase labeled with either C-14 bicarbonate or U-C14-acetate, and movement of the label through the cell and into lipids was monitored over a period of 24 h during a light-dark cycle. Results show that acetate incorporates predominantly into polar lipids (phospho- and glycolipids), while bicarbonate distributes almost equally into polar and neutral lipid stores. These results support a biosynthesis model for PULCA involving fatty acid synthesis components from both plastidial and cytoplasmic locations, which is supported by genomic and proteomic data.

AN ULTRASTRUCTURAL EXAMINATION OF CALCIFICATION IN A POLYMORPHIC COCCOLITHOPHORID, *SCYPHOSPHAERA APSTEINII*

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Coccolithophores are the most significant producers of marine biogenic calcite; however, the precise mechanism and control of the calcification process is poorly understood. *Scyphosphaera apsteinii* (Lohmann) produces flat, ovoid cribriliths and vase-shaped lopadoliths with a range of intermediate morphologies. The cell ultrastructure shows typical Haptophyte features with coccolithogenesis similar to that described for other species including *Coccolithus pelagicus* and *Emiliania huxleyi*. Of particular significance is new evidence of the role the reticular body plays in governing coccolith morphology. In contrast to other studied species, this body is situated between the coccolith vacuole and Golgi complex and is specifically associated with formation of central pores in the calcite structure. Results highlight the essential role of intra and inter crystalline organic material in the growth and arrangement of the calcite crystals. *S. apsteinii* secretes fully mature coccoliths which are attached to the plasma membrane via fibrillar material. Time-lapse light microscopy shows secretion of lopadoliths is base first followed by eversion at the cells surface, the process taking between 1–2 min.

EXPERIMENTAL STRATEGIES FOR INVESTIGATING MEMBRANE EXCITABILITY, CALCIUM SIGNALLING AND CELLULAR HOMEOSTASIS IN CHROMALVEOLATES

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The mechanisms that protists use to sense and respond to intracellular and extracellular signals determine their interactions with the environment. With the recent discovery of fast ‘‘animal like’’ action potentials in diatoms (Taylor 2009) there is increasing evidence that membrane excitability and associated calcium signaling processes, which are important cell signaling mechanisms in other organisms, arose early in eukaryote evolution and were retained in several chromalveolate groups. With the exception of a few models, the functional role of membrane excitability in chromalveolates remains obscure. To advance our understanding of sensory biology, cell signaling and intracellular homeostasis in chromalveolates requires the development of experimental approaches that enable real-time measurements of membrane potential as well as intracellular concentrations of ions such as calcium and protons. Here we present the technical challenges, and the most recent methodological advances to understand cell signaling in diatoms, coccolithophores and dinoflagellates. With the development of methods to measure cellular responses of chromalveolates to changing environmental conditions we can gain a better mechanistic understanding of their environmental physiology and functional roles in aquatic ecosystems.

DEVELOPMENTAL SCULPTING OF THE THIGMOTACTIC CILIATURE PATTERN OF *CONCHOPHTHIRUS*

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The ciliature of the holotrichously ciliated *Conchophthirus curtus* is regionally differentiated. Prior work has identified nine stages of cortical morphogenesis. Here we verify and provide details that lead to final pattern formation within the distinctive thigmotactic field region of the opisthe. Dividing cells were fixed, embedded, oriented, and sectioned for electron microscopy. Cortical morphogenesis in *Conchophthirus* represents a ‘‘point-of-no-return’’ process. We were able to distinguish two major phases for development of the opisthe thigmotactic field. Phase I involves formation of a replication band during stages 1–4 by the duplication of basal bodies within all kinetal rows around the midsection of the mother cell. This band eventually separates the presumptive proter from the opisthe. Phase II involves remodeling only within the replication band and only on the left side of the organism within the presumptive opisthe thigmotactic field. Here, beginning at stage 6, one additional round of basal body proliferation produces one additional basal body for each basal body within the presumptive thigmotactic field. This results in the characteristic zig-zag arrangement of basal bodies for the opisthe thigmotactic field of this organism. Each new basal body becomes ciliated by stage 7. The proter inherits the parental thigmotactic field.

A GENOMIC APPROACH TO UNDERSTANDING MUCILAGE SECRETION IN DIATOMS

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We are coming to understand that the ecological importance of diatoms is not limited to primary productivity, as many diatoms produce extracellular polymeric substances (EPS), which are vital components in algal and bacterial ‘‘biofilms’’. Pad, film and stalk formation is seen in many lineages of both centric and pennate diatoms, and several unrelated lineages of pennate diatoms form tubes. While great effort has been made to chemically identify the types of polysaccharides, proteins and other molecules used to create and modify diatom EPS there is still much about the process we do not know. Araphid pennate diatoms contain clade providing us a unique opportunity to examine this process; specifically, within the genus *Cyclophora*. A newly-described species (*Cyclophora radiata*), is round and does not produce EPS secretions like the closely-related, chain-forming taxon (*Cyclophora tenuis*), is currently in culture. Our goal is sequence the transcriptome of these taxa and compare them, looking for gene products that might be involved with EPS production and secretion.

PROGRAMMED CELL DEATH-LIKE RESPONSES IN *CHLAMYDOMONAS REINHARDTII*

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Numerous bottlenecks in scalable algal biofuels production exist, and ''pond crashes'' in response to abiotic stressors is chief among them. Feedstock algae in raceway ponds are exposed to numerous stressors, including significant temperature fluctuations and high salinity due to evaporation. Elevated temperatures and high salinity are reported to promote programmed cell death-like (PCD) responses in green algal species of *Dunaliella* and *Chlorella*. In this work, we characterize PCD-like responses in, *Chlamydomonas reinhardtii*, a model green alga. Using a multifactorial experimental design, we investigate not only the single-factor PCD responses to elevated temperature and salinity, but the interaction effects of these stressors as well. To examine the possible role of these stressors in promoting PCD, morphological features and intracellular molecular species will be monitored, as will key enzyme activities (e.g. caspases) and changes in expression patterns of loci predicted to have roles in PCD. This area of research will yield biomarkers to monitor pond health and productivity.

CHRYSOCHROMULINA SP.: A MODEL ALGA FOR ANALYZING LIPID BODY BIOGENESIS

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Although microscopic analysis of algal cells has documented the presence of lipid inclusions in many algal species, these inclusions have only recently been recognized as true sub-cellular organelles. Initially, lipid bodies were thought to be static vesicles that served as inert oil-containing storage sites within the cell. New research shows these organelles play a central role in maintaining energy homeostasis. *Chrysochromulina* sp. (Haptophyceae) has been chosen as a model system for probing the life history and lipid composition of this dynamic organelle in chromalveolates. The small (~4 μm) unicellular *Chrysochromulina* sp. has two lipid bodies that lie nestled between its two chloroplasts within the anterior region of the cell. Using the vital fluorescent dye BODIPY 505/515 that accumulates in lipid bodies via a diffusion trap mechanism, we demonstrate that lipid body size changes dramatically when cells are sampled during a 12-h light:12-h dark synchronous growth cycle. Also GC/MS analysis demonstrated that changes in fatty acid profiles occur in these synchronous cultures. Cellular lipid content also reflects physiological cues (e.g., culture age) experienced by the organism.

PHYLOGENETIC ORDER DIFFERENTIATES FATTY ACID COMPOSITION OF 40 NE PACIFIC MACROPHYTES

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NE Pacific macrophyte communities are a diverse mix of species representing four divisions that can confound traditional stable isotope approaches to food web ecology. Fatty acids (FA) may offer a more taxon-specific approach to tracing specific carbon sources, but the distribution of FA among the diverse marine macrophyte groups is poorly characterized. We conducted a phylogenetically diverse survey of the FA content of 40 subtidal and intertidal macrophyte taxa within the Anthophyta, Chlorophyta, Ochrophyta, and Rhodophyta (21 orders) found in the San Juan Archipelago, WA. FA composition of macrophytes differs significantly among phylogenetic orders (perMANOVA, $P = 0.001$). NMDS analyses suggested that orders with more recent origins exhibit more FA variation among species than more ancient orders. We identify several FA which may be useful as biomarker indicators in marine food web studies due to their presence in select taxa within the division and absence in other divisions (e.g., 16:4n-3 in Chlorophyta). We show that the proposed biomarker arachadonic acid (20:4n-6) is not indicative of brown algae in the Ochrophyta only and is present in relatively similar amounts in several Rhodophyta taxa.

CHRONIC EXPOSURE REDUCES ACUTE DOMIC ACID TOXICITY IN VITRO: ROLE OF GLUTATHIONE

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Domoic acid (DomA) is a potent marine neurotoxin. Low concentrations of DomA (100 nM) induce oxidative stress-mediated apoptotic cell death in neurons. In the present study we investigated the effects of prolonged (10 days) exposure of cerebellar granule neurons (CGNs) to a very low concentration (5 nM) of DomA on the acute neurotoxicity of this toxin. CGNs from wild-type mice and from mice lacking the glutamate cysteine ligase modifier subunit (Gclm -/-) were utilized for this purpose. CGNs from Gclm -/- mice have very low glutathione (GSH) levels and are very sensitive to DomA toxicity. In CGNs from wild-type mice, prolonged exposure to 5 nM DomA did not cause toxicity, but reduced apoptotic cell death induced by acute exposure to DomA (100 nM). This protection was not observed in CGNs from Gclm -/- mice. Prolonged DomA exposure increased GSH levels in CGNs of wild-type, but not of Gclm -/- mice. These results indicate that a prolonged exposure to very low levels of DomA can upregulate cellular defense mechanisms that afford partial protection toward a higher acute DomA exposure (Supp. by NSF).

MARINE PLANT PRODUCTION FOR HIGH VALUE APPLICATIONS IN FUNCTIONAL FOOD AND COSMECEUTICAL APPLICATIONS: SECURITY OF SUPPLY AND TRACEABILITY

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As a pioneer in land-based cultivation technology and possessing the largest, on-land commercial macroalgal production facility in the world, Acadian Seaplants Limited (ASL) ensures its cultivated marine plants have an established chain of custody. Wild harvested seaweeds are derived from marine environments exposed to a variety of human influences, making them unreliable and inconsistent sources of high quality compounds. Acadian Seaplants' global leadership in generating pure cultures of marine plant biomass necessitates the integration of reliable traceability systems together with experienced operations management. To accomplish important commercial goals, production protocols have been optimized to batch sizes and critical control points correlated throughout the tightly-monitored growth systems. Extensive documentation is maintained from beginning to end of each production cycle, ensuring security of supply through a safe and traceable system. Detailed information accompanies the product throughout its life span up to and including the required finished product format. The nature of ASL's traceability system inspires commercial confidence and its sustainable cultivation system provides flexibility in addressing customers' unique product requirements while maintaining safety, quality, and environmental stewardship.

LASER IN-SITU SCATTERING AND TRANSMISSOMETRY AS A TOOL TO DETECT HARMFUL ALGAL BLOOMS

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Harmful algal blooms (HABs) are a lasting problem in Texas coastal waters. The blooms are variable in time and space causing difficulty in their management and detection. Four HAB species, *Aureoumbra lagunensis, Karenia brevis, Karlodinium veneficum*, and *Prorocentrum minimum*, were grown in triplicate batch cultures. A laser in-situ scattering and transmissometry (LISST) instrument was used to characterize the shape of each HAB species by detecting the particle size distribution (PSD) in 32 size bins in the range of 2.72 μm to 460 μm. The LISST was used as a low cost, low effort method for detecting HABs in natural water. Each HAB species has a different, distinct PSD showing that the LISST can be used successfully in laboratory conditions. *K. brevis* was detected in natural seawater at 10% of a bloom concentration. These data show that the LISST is potentially a viable instrument for detecting HABs off the Texas coast.

IMMUNOFLUORESCENCE LOCALIZATION OF DIMETHYLSULFONIOPROPIONATE (DMSP) IN THALLI OF THE MARINE MACROALGA, *ULVA LACTUCA*

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Dimethylsulfoniopropionate (DMSP) serves as an anti-herbivore defense molecule in many species of ulvoid macroalgae via its primary breakdown products: dimethylsulfide and acrylic acid. We have recently generated a rabbit polyclonal antiserum against DMSP conjugated as a hapten to bovine serum albumin (BSA) via a carbodiimide-type linker reaction. In the present study, we have tested the usefulness of this antiserum in the specific localization of DMSP in thalli of a known DMSP-producing ulvoid macroalga, *Ulva lactuca*. Initial attempts to localize DMSP in whole-mounted thalli were marginally successful, but compromised due to probe penetration difficulties. We thus developed an indirect immunofluorescence protocol for localizing DMSP in semithin (0.5–1.0 μm) plastic sections of embedded thalli. Such sections provided high resolution images revealing the localization of DMSP (in conjugate form) primarily within the cytosol, and to a lesser degree within the central vacuole and pyrenoids. In some samples, DMSP was also localized within cell walls of the distromate thalli of *U. lactuca*, perhaps as the result of released DMSP being trapped there during fixation. Our results confirm the specificity of the antiserum and suggest it may be useful in studying the production and release of DMSP by ulvoid macroalgae exposed to different environmental conditions.

PUTATIVE ROLES OF MICROCYSTINS ISOLATED FROM *MICROCYSTIS AERUGINOSA* (CYANOBACTERIA) ON HETEROTROPHIC BACTERIOPLANKTON ISOLATED FROM THE ST. JOHNS RIVER (FL)

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Over the last decade, Northeast Florida has undergone a marked increase in blooms of the toxin producing cyanobacterium *M. aeruginosa*. While the negative impacts of microcystins are well known, the true functional roles of these toxins remain unclear. The purpose of this study was to examine the effects of microcystins on co-occurring heterotrophic bacterioplankton commonly found in the St. Johns River. Twenty seven unique bacterial isolates (based on colony morphology) were isolated from river water samples. Isolates were challenged by exposing them to toxin-containing *M. aeruginosa* exudate. Using a disk-diffusion assay, fourteen isolates were inhibited after 24 h, while 13 never elicited a zone of inhibition. The 13 non-inhibited strains were subsequently cultured with microcystins and growth rates accessed via spectrophotometric analysis over one week. Six strains showed significantly ($P < 0.05$) greater growth yields compared to controls that did not contain microcystins, while the other seven stains were not significantly ($P > 0.05$) different compared to controls. In conclusion, it appears that microcystins may stimulate some heterotrophic bacteria while inhibiting others, perhaps leading to a competitive advantage for toxic producing strains.

STRAIN VARIATION IN EUGLENOPHYCIN SYNTHESIS BY THREE CLONES OF *EUGLENA SANGUINEA*

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It is well established that clonal differences exist in algal growth rates, pigment content, and toxin content. Three clones of *Euglena sanguinea* isolated from NC (site of the first recorded fish kills), Texas (site of the second recorded fish mortality event), and from a lake in Ecuador were grown in batch cultures. Clonal isolates were grown in AF-6 media using 35 μmol PAR on a 14:10 L:D cycle at 30 °C. Samples were collected at days 0, 1, 2, 3, 4, 6, 10, 14, 18, 24, and 30 for pigment content (HPLC), cell number and toxin content (HPLC-MS). Growth rates exceeded 0.46 doublings/day for all clones using pigments and cell number data. Toxin content varied by 100-fold between clones.

STABLE ISOTOPE ANALYSIS AND UNDERWATER VIDEO SHOW IMPORTANCE OF HIGHLY LOCALIZED FACTORS IN CONTRIBUTING TO EXCESSIVE GROWTH OF HARMFUL MACROALGAL BLOOMS

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Blooms of ulvoid algae may out-compete other primary producers, cause anoxia, and release toxic hydrogen sulfide fumes as they decay. Underwater video analysis in Washington State, USA indicates that ulvoid algal high on average state-wide in a given year, yet many sites will show declines during that year and vice versa. We examined nitrogen availability and sources using stable isotope analysis at a focus site and two control sites. Most ulvoid algae in Puget Sound are not nitrogen limited; rather light and desiccation on steeply-pitched shorelines control maximal abundance. At our focus location in Dumas Bay, we found that algae appeared nitrogen-limited since the intertidal is extensive yet kept constantly moist by streams and groundwater. Algae growing in most of Dumas Bay had an isotopic signature similar to that seen at control sites and consistent with upwelling-driven sources. At the mouth of one creek discrepancies in this ratio suggest an anthropogenic source of nitrogen. We conclude that differences in upwelling and summer weather are not expected to uniformly affect all sites across the state accounting for the interaction effect between sites and year of study seen in underwater video analysis.

MORPHOLOGICAL CHARACTERIZATION AND SECONDARY METABOLITES OF A SYMBIOTIC CYANOBACTERIUM STRAIN GI1 ISOLATED FROM THE MARINE SPONGE *TERPIOS HOSHINOTA*

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Terpios hoshinota, a cyanobacteriosponge, caused the coral ''black disease'' at Green Island off the southeastern coast of Taiwan. From *T. hoshinota*, a cyanobacterium (GI-1) was isolated, and axenically cultivated in the lab. The GI1 was supposedly a new cyanobacteria species as demonstrated by the 16S rDNA sequence and its unique morphological characteristics. The phylogenetic analysis revealed that the GI1 was taxonomically corresponded within the order Pleurocapsales. However, the maximum identity was only 94% between GI1 and *Pleuocapsa*, *Myxosarcina* and *Dermocarpa* strains, respectively. The GI1 was examined for thylakoid structure and cell division type with TEM as well as traditional morphology with SEM and LM. Cells of GI1 were able to divide by binary. The formation of endospores and positive phototaxis of released endospores were observed in GI1.he major fatty acids, C16:0 and C16:1, were present 98.3% of total fatty acids. One new and a known cyclic tetrapeptide (1–2), and ten diketopiperazines (3–12) were isolated from the culture medium of GI-1. Eight of ten diketopiperazines were proline-containing cyclic dipeptides. The structures of 1–12 were elucidated by NMR and mass data.

REGENERATION FROM THALLUS FRAGMENTS OF A RED ALGA *GELIDIUM* SPECIES

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Gelidium is an excellent source of high quality agar, and there is a great need of cultivation to increase biomass because most of *Gelidium* species are very slow in growth rate and difficult to cultivate in fishery farms. We found a *Gelidium* species in Korea that is small with a size of up to 5 cm, and have ''repair'' bands on branchlets. We compared regeneration of apical and subapical fragments of about 5 mm in size through culture. Cultures were maintained in f/2 medium at 20 °C. All cultures were kept a low light intensity during the first week. Up to 20 primary branches arose from each fragment after 4 weeks of culture, and up to 60 secondary branches from each fragment were produced after 7 weeks of culture. More regenerated branches were likely produced from basal side than apical side. After 13 weeks, regenerants grew up to 5 cm and became a typical thallus of *Gelidium*. Our regeneration experiment suggests a capability of rapid regeneration of a species with repair band that contributes to mass cultivation of *Gelidium* for biotechnology.

BIOCHEMICAL ENGINEERING OF *TETRASELMIS CHUII* FOR COMPROMIZING BETWEEN HIGH CELL DIVISION AND LIPID CONTENT

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A comparative study was carried out on a variety of batch-wise grown microalgae to explore the fastest grown and highest lipid producer candidate towards the biofuel invention. The results showed that although *Tetraselmis chuii* was the best grown microalga under basal cultivation conditions, it was the least lipid producer among the examined species. A linear correlation between the alga's cell size and the lipid content was demonstrated. However, after being transferred into N-deficient nutrient medium, *T. chuii* cells were equally sized and attained the maximum size after 15 days of cultivation. In addition, the lipid produced by the alga increased by about 35% compared to the basal growth medium. The study demonstrated that biochemical engineering of *T. chuii* using N-deficient growth medium condition could compromise between cell division and lipid production.

QUANTITATIVE GENE EXPRESSON OF PROTEINS ASSOCIATED WITH SYNTHESIS OF NEUTRAL LIPID AND LIPID BODIES IN THE HAPTOPHYTE *EMILIANIA HUXLEYI*

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E. huxleyi, a globally important coccolithophorid, synthesizes an unusual set of PolyUnsaturated Long-Chain Alkenones, Alkenoates, and Alkenes (PULCA) as its neutral lipid. These lipids are stored in lipid bodies (LBs) (Eltgroth 2005), much as the triacylglycerols produced by most other algae as neutral lipid, and are structurally attractive as biofuels. Prior work has shown PULCA accumulation under nutrient limitation (Prahl 2003), and a proteomics study of E. huxleyi LBs and associated endomembranes identified a number of low-abundance proteins likely involved in neutral lipid biosynthesis and catabolism (Wolfe and Erlendson manuscript in preparation). I am quantifying expression of these genes using RT-PCR under different growth conditions, including growth phase, light vs. dark, bicarbonate addition, and lipid synthesis inhibitors. Here, I present results of qPCR method development and data on gene expression levels over a growth phase.

ULTRASOUND ASSISTED EXTRACTION OF LIPIDS FROM MICROALGAE

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The goal of the study was to enhance extraction of lipids from microalgae using ultrasonic treatment. Dewatered and frozen samples of microalgae were freeze-dried and mixed with the solvent (n-hexane: isopropanol in ratio 3:2) prior to ultrasonic treatment. A solid probe resonating at maximum amplitude of 124 nm was used to treat the sample and applied amplitudes ranged from 85% to 40%. Treatment time was also varied from 1 to 4 h. After treatment, lipids were extracted with the same solvent followed by phase separation using sodium sulphate. The lipid yield was then assessed by gravimetric method and the results were subjected to statistical analysis. The fatty acid profile of the extracted lipid was obtained using gas chromatography and the free fatty acid content was calculated. The result showed a significant increase in lipid extraction following ultrasound treatment. Both ultrasound amplitude and treatment time have significant effect on lipid yield. The relationship between the two factors was not completely proportional. It was demonstrated that ultrasound treatment could be an efficient method of cell disruption for lipid extraction from microalgae.

ANALYSIS OF *CHROMULINA FREIBURGENSIS* - LIPIDS & SACCHARIDES FOR THE USE OF BIOFUELS

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Algae are being investigated world-wide as a source for biofuels. We are studying the algal species *Chromulina frieburgensis* Defl., a single-celled, golden brown algae in the class Chrysophyceae. Isolated from acidic mine waste waters, this alga contains large oil stores in its cell vacuoles as noted by microscopy. Extracts of the dried alga and the dried alga that had been grown in a modified acid medium were reacted with a sulfuric acid-methanol solution to form esters of any fatty acids (FAMEs). GC/MS analysis of the derivatized extract revealed an extremely complex mixture that eluted at relatively high temperatures but no detectable FAMEs. Trans-esterification of the dried alga produced a number of C16 and C18 FAMEs. A water extraction procedure produced a number of compounds, including various glucans that were analyzed by LC/MS. Results on algal growth rates and extractable oil composition resulting from the variation of environmental factors such as temperature, light intensity, and growth media will be presented.

CIGUATERA FISH POISONING IN THE CARIBBEAN

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Tropical dinoflagellates of the genus *Gambierdiscus* cause ciguatera fish poisoning (CFP) in humans through ingestion of fish that have bioconcentrated ciguatoxins. CFP is the most commonly reported marine toxin related illness and it can be fatal. CFP occurs circumtropically and is endemic to the Caribbean where it correlates positively with water temperature. Consequently, there is increasing concern that higher temperatures associated with climate change could broaden and increase incidences of CFP. This concern prompted examination of water temperatures in the Caribbean for areas that could sustain rapid *Gambierdiscus* growth rates year-round (> 29 °C, see Kibler et al. abstract). Long-term sea surface temperature (SST) and buoy data from the region indicate the number of days with SSTs > 29 °C has nearly doubled (44 days to 86 days) in the last three decades. Our results show that areas of high, stable water temperatures in the eastern Caribbean correlate with the highest CFP incidence rates. Modeling efforts are currently underway to determine how decadal and longer warming trends will affect *Gambierdiscus* growth rates, habitat range and, potentially, CFP occurrence rates.

USING A CYST RECORD TO ASSESS HISTORICAL RELATIONSHIPS BETWEEN THE HARMFUL ALGA *ALEXANDRIUM* SP. AND CLIMATE IN SEQUIM BAY, WASHINGTON

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Detection of paralytic shellfish toxins (PSTs) due to blooms of the harmful alga *Alexandrium* sp. has increased in Puget Sound, Washington, since the 1970s. This increase has been linked to large-scale climate variability such as the Pacific Decadal Oscillation (PDO), and local variables such as air temperature, stream flow, and sea surface temperature. However, existing records, based on PSTs in shellfish, are relatively short, dating only to 1957, and therefore it is difficult to statistically assess the influence of short-term, stochastic environmental variability versus long-term trends in relation to climatology. *Alexandrium* sp. exhibits an alternation between a motile, free-living, vegetative stage with a non-motile, benthic, cyst stage. Hence, we examined the relationship between historical climate variability and profiles of *Alexandrium* sp. cysts in a sediment core from Sequim Bay, WA. Our results suggest that there is no statistically significant relationship between the cyst record and PDO or stream flow, but there is a positive, significant relationship between local air temperature and sea surface temperature, indicating that local environmental variability is an important controller of *Alexandrium* sp. populations.

DISCOVERY AND DISTRIBUTION OF *MEDIOPYXIS HELYSIA* KÜHN, HARGRAVES & HALLIGER IN BREIÐAFJÖRÐUR, WEST ICELAND

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A survey on phytoplankton abundance, community composition and physical and chemical drivers was initiated in Breiðafjörður, West Iceland, in May 2007. In the same year a new species of diatoms to Icelandic waters, *Mediopyxis helysia*, was detected in the phytoplankton community. The species has been observed in Breiðafjörður annually from 2007 to 2011 and has been detected sporadically at 10 sample locations. *M. helysia* was frequently detected in autumn and winter with erratic occurrence in spring and summer. Comparison of *M. helysia* abundance with measured environmental parameters revealed no significant correlation. In Iceland, *M. helysia* has only been detected from Breiðafjörður and its origin and dispersal mechanism is unknown. Currently, the locations of possible origin are in the North Sea (Scotland, German Bight) or from the east coast of North-America (Gulf of Maine, Bay of Fundy), the only locations in the world where the species has been reported hitherto. *M. helysia* is a large and conspicuous species which is hard to overlook and thus it might be indicative of more extensive transport of inconspicuous phytoplankton or other marine microscopic organisms.

THE EFFECTS OF ELEVATED PCO$_2$ ON THE PHYSIOLOGY OF COCCOLITHOPHORE *EMILIANIA HUXLEYI*

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As anthropogenic CO$_2$ is added to the atmosphere, CO$_2$ dissolves into the ocean and lowers the pH of seawater. Calcifying marine organisms, including coccolithophores, such as *Emiliania huxleyi*, may be sensitive to these changes in ocean chemistry. We investigated the effect of increased pCO$_2$ on two isolates of *Emiliania huxleyi*: the calcifying strain, CCMP 2668, and the non-calcifying strain, CCMP 374. These experiments were performed in enclosed experimental chambers with target CO$_2$ levels of 380 ppm (present day), 760 ppm (2x present day) and 1000 ppm (predicted year 2100 level under IPCC ''business as usual'' scenario) through an atmospheric gas exchange system. Semicontinuous cultures were maintained for 11 days, during which time cultures were regularly sampled for particulate dimethylsulfoniopropionate (pDMSP), particulate organic and inorganic carbon, cell size, growth rate, and chlorophyll content. Analysis of these samples is currently underway.

ANALYSIS OF *EUTREPTIELLA* TRANSCRIPTOME USING 454 GSFIX TITANIUM

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To understand the genetic network and environmental factors regulating various ecological and physiological processes critical for controlling energy flows in the marine ecosystem, it is necessary to study genome-scale profiling of gene expression for phytoplankton. In this project, we used 454 sequencing technique to investigate gene expression profiles of a common estuarine microalgae, *Eutreptiella* sp. from Long Island Sound. About 4×10^6 reads were yielded in two 454 sequencing runs including contrasting light and nutrient conditions. The most abundant genes found were associated with metabolic process, cellular process, biological regulation, and cellular component organization. Under the light condition, genes were more diverse than those under the dark condition. The results suggest that cell activity was more complex under the light condition. The genes differentially expressed under different conditions and their possible functions are discussed.

EXAMINING RNA-PROCESSING IN MICROSPORIDIA WITH HIGH-THROUGHPUT TRANSCRIPTOME SEQUENCING

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Microsporidian parasites of the genus *Encephalitozoon* have the smallest eukaryotic nuclear genomes. The genome of *E. cuniculi* is a mere 2.9 Mb, encodes approximately 2000 protein-coding genes, and has very few, short introns. We have shown that RNA-processing in these parasites is unusual in several respects; multigene transcripts are produced, introns are actively spliced in the proliferative stage only, and 5' untranslated regions are the shortest known. RNA extracted from proliferative stage cells was sequenced by Illumina mRNA-seq, producing 100 bp reads. By aligning these reads to the *E. cuniculi* reference genome, we are able to assess intron splicing efficiency, gene expression levels, the frequency and location of SNPs, as well as discovering novel splice sites. Our preliminary findings show that transcription levels are highly variable between genes and there is a significant amount of transcription in non-coding regions. Also, intron splicing efficiency is quite low on average, with many more transcripts containing introns than having their introns spliced. However, a few introns are spliced at significantly higher efficiency, which may be related to high levels of transcription.

ALGIVOROUS AMOEBAE FROM TROPICAL MARINE WATERS

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Benthic gymnamoebae (''naked'' amoebae) are often abundant and productive consumers in marine ecosystems, but little is known about their diversity or biology, especially of algivorous species in tropical climates. Here, data are provided on the morphology, taxonomy, environmental tolerances, specific growth rates, grazing rates, and prey choices of several species of algivorous amoebae isolated from Hawaiian waters, with some comparison to species occurring in the temperate waters of the northeast Pacific and northwest Atlantic. Most species observed in Hawaiian waters belong to genera of the paramoebid clade (*Mayorella*, *Neoparamoeba*, *Paramoeba*), though species belonging to other lineages are represented. Some of the species have not been found in temperate waters and may be restricted to the tropics. Most species are facultative algivores, surviving also on bacteria. Individual amoebal species prefer some prey species (algae and bacteria) over others, and prey preferences differ from species to species. Data on population dynamics of amoebae in two-component laboratory cultures (one amoeba, one prey) have contributed to the formation of a preliminary model for contamination dynamics in algal production bioreactors and ponds.

CORRELATED MORPHOLOGICAL AND MOLECULAR DATA ESTABLISH THE POLYPHYLY OF NANODIATOMS PREVIOUSLY ASSIGNED TO *NANOFRUSTULUM SHILOI*

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The genus *Nanofrustulum*, containing the single species *N. shiloi*, was created to describe small, marine, chain-forming, araphid pennate diatoms isolated from water samples as well as from foraminifera, where they are present as endosymbionts. All who have studied *N. shiloi* have found considerable variation among specimens in the diatom's morphological features. This variation has been taken to mean that *N. shiloi* is a ''very polymorphic'' species. Here, we establish that differences in frustule structure among strains of *Nanofrustulum*-like diatoms from Hawaiian waters are correlated with differences among the strains in 18s rRNA gene sequences. Phylogenetic trees based on these sequences establish that ''*Nanofrustulum*-like'' diatoms are not one species but several, and that these species do not form a clade, but instead are scattered among the ''staurosiroid'' pennates – as is the case for most of the rest of the ''staurosiroid'' genera, *Nanofrustulum* is polyphyletic. From the large number of variants described in the literature, there may be many more species of ''*Nanofrustulum*-like'' diatoms than are reported here – from among which, the 'real' ''*N.*'' *shiloi* may be difficult to pick out.

LIGHT AND SCANNING ELECTRON MICROSCOPE OBSERVATIONS OF THE DIATOM SPECIES *COSCINODISCUS ALBORANII* AND *HASLEA WAWRICKAE* IN THE NORTHEASTERN GULF

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We are currently re-assessing the state of the diatom communities in the northeastern Gulf of Mexico in response to the recent oil spill. In recent net plankton (25-μm mesh) collections from St. Joseph's Bay, Florida, two distinctive warm water planktonic forms were recognized: *Coscinodiscus alboranii* and *Haslea wawrickae. C. alboranii* is distinguished from other members of the genus by the presence of endochiastic areolae along the margin and by the presence of two types of vela covering valve-face areolae. These unique features suggest it occupies an anomalous position within the redefined genus. This species was common in near shore samples collected in October 2010; this represents the first record of the species from the northeastern Gulf of Mexico. *Haslea wawrikae* is a naviculoid diatom with long (> 300 μm), narrow (4–5 μm) spindle-shaped valves with produced ends. It was widely distributed in St. Joseph's Bay in October 2010; this is the second record of the species within the bay. The presence of *H. wawrickae* at multiple stations suggests it plays an important role in this shallow marine ecosystem.

EFFECTS OF THE ANTHROPOGENIC APRIL 2010 BP DEEPWATER HORIZON CRUDE OIL SPILL ON SEAWEED DIVERSITY IN THE NW GULF OF MEXICO

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Two 5-day ship-based dredging expeditions centered on previously studied hard banks throughout the Gulf to assess the impacts of the Deepwater Horizon oil spill on the diversity, vitality, and distribution of offshore seaweeds living principally at 45–90 m depth. Results analyzed from the first 5-day leg of the expedition offshore Louisiana, Mississippi and Alabama (December 2010) indicate that macroalgal diversity of the previously documented assemblage appeared limited in all dredged sites. The six species of seaweeds dredged typically have a zone of meristematic surface cells or are embedded in a gelatinous matrix. Strong petroleum odors were pervasive when the dredge was retrieved from ~55 m depth at Ewing Bank 2 (Fish Haven) far west of the Deepwater Horizon Explosion site; samples included individual carbonate nodules covered by light crude oil. The material is awaiting final HC fingerprinting analysis. In the second leg of the RAPID expedition, April 2011, seaweed diversity had increased to ~20 species. Additional seasonal dredging trips are urgently needed to fully assess whether the marine flora shows evidence of recovering to pre-event summer levels.

LINKING SEA ICE ALGAE TO BENTHIC AND PELAGIC COMMUNITIES: SINKING RATES AND NUTRITIONAL QUALITY OF ORGANIC SUBSTANCES EXPORTED FROM SEA ICE

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Organic matter pools accumulate within first year Arctic sea ice during blooms of ice algae. These ice algae are responsible for a significant fraction of annual polar marine primary production. Upon summer melt these large deposits of organic material, as well as the ice algae themselves, are exported from the ice. However, the ecological and biogeochemical connection between exported ice material and the underlying pelagic and benthic communities remains relatively unknown. The sinking rates and nutritional qualities of particulates exported from the ice are likely to be key factors. We hypothesize that sinking rates of particles released from melting sea ice will vary in relation to the composition and biomass of the ice community and the overlying snow cover. To test this hypothesis, we collected sea ice cores from under various snow cover depths along the Chukchi Sea near Barrow, AK. The ice cores were sectioned into three distinct 10 cm. segments (0–10 cm, 10–20 cm from the bottom, and 50–60 cm from the top). These sections were diluted in filtered seawater, melted at 5 °C, and the mean particle sinking velocities were quantified using a SETCOL experimental design. Results indicate that there is a strong spatial and temporal change in particle size and sinking velocity based on both the biomass of the ice community and overlying snow depth. These trends indicate a potential change to dietary quantity and quality as well as temporal availability of algal produced organic matter with respect to diminishing ice cover and earlier ice breakup.

TAXONOMIC STUDY ON A NEW NAKED THAUMATOMONAD (PHYLUM CERCOZOA)

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Thaumatomonads (phylum Cercozoa) are bacteriovorous flagellates commonly found in marine and freshwater environments. They are characterized by possessing many siliceous scales on their cell surface. The shape of the scale is considered to be an important characteristic for their classification and over 28 species (7 genera) have been described so far. In the present study, we report a new colorless protist (the strain YPF708) that was found in a sand sample collected at a beach in Motoyoshi, Japan. The cells were teardrop shape with two unequal flagella (ca. 4 and 17 μm), and showed 5–11 μm in length. Phylogenetic analysis using 18S and 28S ribosomal DNAs showed that the YPF708 formed a clade with thaumatomonads with high statistical support. However, ultrastructural observations using an electron microscope revealed that the YPF708 lacked siliceous scales and possessed a unique microbody. Combining both molecular and morphological data, we concluded that the YPF708 should be treated as a new genus and species of Thaumatomonadida and it would be reasonable to consider that the siliceous scale was secondarily lost in the YPF708 lineage.

A SURVEY OF PITFALLS IN PARABASALID DIVERSITY AND PHYLOGENY IN THE HINDGUT OF LOWER TERMITES

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Parabasalids are flagellates that commonly associate with animal hosts and have undergone an explosion of diversity within wood eating cockroaches and lower termites. We have identified key issues in the study of these organisms. Prior to examining the protists it is important to identify their host by molecular barcoding as morphology alone is often not sufficient. For example, we recently examined the previously unstudied hindgut content of a lower termite and identified what would appear to be a new species of *Pseudotrichonympha*. However, host barcoding revealed mistakes in termite taxonomy, what might have been a new *Pseudotrichonympha* species is really the type species of this genus. Similarly, many protist species, e.g. *Trichonympha agilis*, are thought to be found in many termites. This is not the case: we show that ''*T. agilis*'' in different termites are in fact distant relatives. We also demonstrated the inadequacy of morphology for genus and species level delineation of termite symbionts. Two genera, *Coronympha* and *Metacoronympha* are actually a single genus, containing life cycle stages of the same organism. Host identification is paramount to symbiont identification.

DEVELOPMENT OF SEAWEED CULTURE SYSTEM TECHNOLOGIES TO SUPPORT INTEGRATED MULTI-TROPHIC AQUACULTURE AND SEA VEGETABLE AQUACULTURE IN NEW ENGLAND COASTAL WATERS

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The marine aquaculture industry in the United States has been mainly restricted to finfish and shellfish, and growth has been limited due to environmental and social sustainability concerns. Seaweed aquaculture represents a bioextractive solution (=bioremediation) to excess inorganic nutrients generated in coastal waters, while also providing biomass for biofuels, food, phycocolloids, and other valuable phycoproducts. The development of a seaweed aquaculture industry in the United States could be important for the growth of other cultured species through integrated multi-trophic aquaculture (IMTA), sea vegetable production, or bioremediation of coastal waters. By adapting techniques developed in Asia for our native varieties of kelp and *Gracilaria*, we aim to develop nursery culture systems for commercial scale mass production of young seaweeds, and then transfer this technology to the commercial sector. We have developed a scalable culture system for our native sugar kelp, *Saccharina latissima* (Linnaeus) C.E.Lane, C.Mayes, L.Druehl & G.W.Saunders, and are currently culturing young plants both in the Gulf of Maine and in Long Island Sound. The next phase of the project will be focused on *Gracilaria tikvahiae* McLachlan, a potential commercially important species of red algae in southern New England.

KWAKWAKA'WAKW TRADITIONAL ECOLOGICAL KNOWLEDGE OF *PORPHYRA ABBOTTIAE*

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I evaluated the hypothesis that adopting modern technology in the lheqqesten (*Porphyra abbottiae* Krishnamurthy) harvest has improved the survival of the harvest. The Kwakwaka'wakw of northern Vancouver Island and the adjacent mainland have harvested, traded, and consumed lheqqesten as both medicine and food for centuries. Sociological upheavals including residential schools, a wage-based economic system, and market foods have disrupted the transmission of traditional ecological knowledge (TEK) from elders to younger generations. I compared ethnological records from the early 20th century to TEK from interviews and harvesting expeditions conducted with Kwakwaka'wakw elders and cultural specialists. I found that the basic process for the preparation of lheqqesten, from harvesting to consumption, has changed little over the past century. I also found that few aspects of TEK have become redundant from the adoption of new tools. I conclude that incorporating new tools into the harvest helps contemporary harvesters circumvent time and weather-related barriers to harvesting lheqqesten, improving the harvest's survival.

SEQUENCING THE ALGAL TREE OF LIFE

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Algae, defined as photosynthetic eukaryotes other than plants, constitute a major component of fundamental eukaryotic diversity. Acquisition of the ability to conduct oxygenic photosynthesis through endosymbiotic events has been a principal driver of eukaryotic evolution, and today algae continue to underpin aquatic food chains as primary producers. Algae play profound roles in the carbon cycle, can impose health and economic costs through toxic blooms, and are candidate sources for bio-fuels; all of these research areas are part of the mission of DOE's Joint Genome Institute (JGI). To date JGI has sequenced, assembled, annotated, and released to the public the genomes of 15 species and strains of algae, sampling most of the major clades of photosynthetic eukaryotes. With more algal genomes currently undergoing analysis, JGI continues its commitment to driving forward basic and applied algal science.

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