



THE PHYCOLOGICAL SOCIETY OF AMERICA



The *Phycological Society of America* (PSA) was founded in 1946 to promote research and teaching in all fields of Phycology. The society publishes the *Journal of Phycology* and the *Phycological Newsletter*. Annual meetings are held, often jointly with other national or international societies of mutual member interest. *Phycological Society of America* awards include the **Bold Award** for best student paper at the annual meeting, the new **Student Poster Award** for the best student poster at the annual meeting, the **Provasoli Award** for outstanding papers published in the *Journal of Phycology*, and the **Prescott Award** for the best Phycology book published within the previous two years. The society provides financial aid to graduate student members through **Croasdale Fellowships** for enrollment in phycology courses at biological stations, **Hoshaw Travel Awards** for travel to the annual society meeting, and **Grants-In-Aid** for supporting research. To join the *Phycological Society of America*, contact the membership director. Society Webpage: <http://www.psaalgae.org/>

LOCAL ORGANIZER FOR 2007 PSA/ISOP ANNUAL MEETING: Glen Thursby, *US Environmental Protection Agency*

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THE INTERNATIONAL SOCIETY OF PROTISTOLOGISTS



The *International Society of Protistologists* (ISOP) is an association of scientists devoted to research on single-celled eukaryotes, or protists. The ISOP promotes the presentation and discussion of new or important facts and problems in protistology, and works to provide resources for the promotion and advancement of this science. The Society publishes the *Journal of Eukaryotic Microbiology*, an electronic newsletter, *The Stentor*, and special publication on protists, including *The Illustrated Guide to the Protozoa*. Awards of the International Society of Protistologists include the **Hutner Award** for outstanding contribution to protistology by young investigators, the **Jahn-Bovee Award** for best student presentation at the annual meeting, the **Corliss Ciliate Systematics Award** for best publication in the field during a 2-year period, and the **Trager Award** for best publication in a volume of the *Journal of Eukaryotic Microbiology*. The **Holz-Connor Travel Fund** provides financial assistance for students and young investigator to attend the annual meeting. To join the International Society of Protistologists and learn more about its activities go to <http://www.uga.edu/~protozoa/>.

ISOP EXECUTIVE COMMITTEE

President:	Wilhelm Foissner <i>Universität Salzburg, Austria</i>
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SOCIETY AND AFFILIATES

British Society of Protozoologists (BSSP)
Czech Section (Czech Society of Parasitologists)
East Coast Section (USA)
German Society of Protozoologists
Groupement des Protistologues de Langue Francaise
Società Italiana di Protozoologia
Israel Society for Parasitology, Protozoology and Tropical Diseases
Korean Protozoologists
Russian Society
Scandinavian Section
Ukraine Protistology

Welcome to Rhode Island

Rhode Island is the smallest state in the United States, but it has the longest name. Its official name is the State of Rhode Island and Providence Plantations. It covers an area of only 1,214 square miles. Its distance north to south is 48 miles, and east to west only 37 miles. In spite of its small size, because of Narragansett Bay and its numerous islands Rhode Island has a total linear coast line of almost 400 miles—which explains why it’s called the Ocean State.

Roger Williams founded the first permanent white settlement in Rhode Island at Providence in 1636 on land purchased from the Narragansett Indians. In doing so he established the first working model of democracy after he was banished from Plymouth, Massachusetts because of his “extreme views” concerning freedom of speech and religion.

At the start of the Revolutionary War, Rhode Islanders were among the first colonists to take action against British rule and on May 4, 1776, Rhode Island was the first of the original thirteen colonies to renounce allegiance to Great Britain and declare independence. Rhode Island’s independent spirit was still evident at the close of the Revolutionary War. It was the last of the thirteen colonies to ratify the U.S. Constitution, demanding that the Bill of Rights, which guaranteed individual liberties, be added. Thomas Jefferson and John Adams both acknowledged Roger Williams, as the originator of the concepts and principles reflected in the First Amendment. Among those principles were freedom of religion, freedom of speech, and freedom of public assembly.

Famous Rhode Islanders

George M. Cohan: Singer, dancer, producer, actor, playwright and composer—the first artist/entertainer to be honored by Congress. In 1936, he received a Congressional Gold Medal in recognition for his patriotic songs *Over There* and *A Grand Old Flag*.

Gilbert Stuart: Foremost painter of portraits of George Washington, John Adams, Thomas Jefferson, James Madison and James Monroe. The Rhode Island state capitol houses the historic painting of George Washington by Gilbert Stuart which appears on the \$1 dollar bill.

Nathanael Greene: Revolutionary War general, second-in-command to George Washington.

Esek Hopkins: First Commander-in-Chief of the Continental Navy.

Anne Hutchinson: The first woman to establish a town in America—Portsmouth, Rhode Island.

Nap Lajoie: The American League’s first batting champion and an inductee into the Baseball Hall of Fame.

Samuel Slater: Father of the American Textile Industry

above mostly from www.visitrhodeisland.com/facts_history/history.aspx

	Grand Ballroom (Salons III, IV & V)	Salons (I & II)	Bristol A&B
MONDAY	<i>Continental Breakfast (6:50-7:50 am) Rotunda</i>		
	PSA/ISOP Joint Symposium on Symbiosis (8:00-9:50 am)		
	<i>Mid-Morning Break (9:50-10:20 am) Grand Foyer</i>		
	PSA/ISOP Joint Symposium on Symbiosis (10:20-11:50 am)		
	<i>Lunch Break: Ticketed (pre-paid) in Rotunda at noon</i>		
	PSA Bold Award 1 (1:30-3:30 pm)	ISOP Ecology/Symbiosis (1:50-3:30 pm)	
	<i>Mid-Afternoon Break (3:30-3:50 pm) Grand Foyer</i>		
	PSA Bold Award 1 (3:50-5:30 pm)	ISOP Ecology/Symbiosis (3:50-5:30 pm)	
	<i>PSA/ISOP Auction and Mixer (7:00 - 9:30 pm) Grand Foyer</i>		

TUESDAY	<i>Continental Breakfast (6:50-7:50 am) Rotunda</i>		
	PSA Special Session: Physiological & Structural Advantages of Chloroplast Evolution (8:00-10:00 am)		ISOP Special Session: Drug Targets in Parasitic Protists (8:00-9:50 am)
	<i>Mid-Morning Break (PSA: 10:00-10:30 am--ISOP 9:50-10:20 am) Grand Foyer</i>		
	PSA Bold Award 2 (10:30 am-12:10 pm)		ISOP: Special Session cont'd & Contributed Papers: Cell Biology/Physiology/Behavior (10:20 am- noon)
	<i>Lunch Break: Ticketed (pre-paid) in Rotunda at noon / PSA Student-Post Doc Lunch in Atrium at noon</i>		
	ISOP Hutner Lecture & ISOP Symposium: Biogeochemical Cycling (1:30 - 3:30 pm)	PSA Contributed Papers: Physiology & Biochemistry (2:30-4:10 pm)	PSA Contributed Papers: Ecology & Population Biology 1 (2:30-4:10 pm)
	<i>Mid-Afternoon Break (ISOP 3:30-3:50 pm: PSA 4:10-4:30 pm) Grand Foyer</i>		
	ISOP Symposium: Biogeochemical Cycling cont'd (3:50-4:50 pm)		PSA Business Meeting (4:30-? pm)
	<i>PSA Student/Postdoc Mixer (8:00 pm - 10:00 pm) Rotunda</i>		

	Grand Ballroom (Salons III, IV & V)	Salons (I & II)	Bristol A&B
WEDNESDAY	<i>Continental Breakfast (6:50-7:50 am) Rotunda</i>		
	PSA Special Session: Phylogenetics, Systematics, & Biogeography of Macroalgae (8:00 - 10:00 am)		ISOP Special Platform Session: Protists & The Molecular & Informatics Revolution (8:10-10:00 am)
	<i>Mid-Morning Break (10:00-10:30 am) Grand Foyer</i>		
	PSA Contributed Papers: Phylogenetics & Taxonomy 1 (10:30-11:50 am)	PSA Contributed Papers: Cellular & Molecular Biology 1 (10:30-11:30 am)	ISOP Special Session Cont'd (10:30-11:50 am)
	<i>Lunch Break: Ticketed (pre-paid) in Rotunda at noon / ISOP Ticketed (pre-paid) Business Lunch in Barrington at 12:30pm</i>		
	PSA Contributed Papers: Phylogenetics & Taxonomy 2 (1:50-3:10 pm)	PSA Contributed Papers: Ecology & Population Biology 2 (1:30-3:10 pm)	
	<i>Mid-Afternoon Break (3:10-3:30 pm) Grand Foyer</i>		
	PSA Contributed Papers: Phylogenetics & Taxonomy 3 (3:30-5:30 pm)	PSA Contributed Papers: Ecology & Population Biology 3 (3:30-5:30 pm)	
<i>PSA/ISOP Poster Session and Mixer (7:00-9:30 pm) Plaza Ballroom & Foyer</i>			

THURSDAY	<i>Continental Breakfast (6:50-7:50 am) Rotunda</i>		
	PSA Special Session: Energetic & Elemental Stoichiometries in Phytoplankton Ecology (8:20-9:50 am)		ISOP Contributed Papers: Diversity/Phylogeny (8:00-9:50 am)
	<i>Mid-Morning Break (9:50-10:20 am) Grand Foyer</i>		
	PSA Contributed Papers: Phylogenetics & Taxonomy 4 (10:20-11:40 am)	PSA Contributed Papers: Cellular & Molecular Biology 2 (10:20-11:40 am)	ISOP contributed Papers: Diversity/Phylogeny cont'd (10:20-noon)
	<i>Lunch Break: Ticketed (pre-paid) in Rotunda at noon</i>		
	PSA Contributed Papers: Phylogenetics & Taxonomy 5 (1:30-3:10 pm)	PSA Contributed Papers: Ecology & Population Biology 4 (1:30-3:10 pm)	ISOP Past President Address & Contributed Papers: Ecology (1:30-3:10 pm)
	<i>Mid-Afternoon Break (3:10-3:30 pm) Grand Foyer</i>		
	Phyco-Speed Dating (3:30-5:00 pm)		ISOP Contributed Papers: Ecology cont'd (3:40-5:00 pm)
<i>PSA-ISOP Banquet & Awards Ceremony (6:00 pm Grand Foyer: 6:30-9:30pm Grand Ballroom)</i>			

SATURDAY, 4 AUGUST

PSA Board Of Trustees Meeting, – Wellington Room, 1:00 to 6:00 PM

SUNDAY, 5 AUGUST

PSA Executive Committee Meeting, Bristol A, 8:30 AM to 6:00 PM

ISOP Executive Committee Meeting, Bristol B, 8:30 AM to 6:00 PM

Opening Social and Mixer, Rotunda, 7:00 to 10:00 PM

MONDAY, 6 AUGUST

6:50 – 7:50 Continental Breakfast in Rotunda

Salon III-V, Monday Morning:

8:00 PSA and ISOP welcoming remarks

PSA-ISOP Joint Symposium: Protistan Symbiosis

Presiding: Charles Delwiche, University of Maryland and D. Wayne Coats, Smithsonian Environmental Research Center

8:20 The distribution of plastids among eukaryotes: past, present, and future

Delwiche, Charles.

Cell Biology and Mol. Genetics, University of Maryland, College Park, MD, USA.

8:50 Organelle retention in the photosynthetic ciliate *Myrionecta rubra*

Johnson, Matthew.

Institute of Marine and Coastal Sciences, Rutgers University, New Brunswick, NJ, USA.

9:20 *Symbiodinium*-cnidarian symbioses: ontogeny and diversity

Coffroth, Mary Alice¹; Voolstra, Christian²; Medina, Mónica².

1. Geological Sciences, University at Buffalo, Buffalo, NY, USA. 2. University of California at Merced, Merced, CA, USA.

9:50 BREAK

10:20 Convergent evolution in eukaryotic cells

Leander, Brian.

Zoology & Botany, University of British Columbia, Vancouver, BC, Canada.

10:50 **Primary and secondary bacterial symbionts of *Euplotes*: a complex net of evolutionary and adaptive phenomena**
Petroni, Giulio.
Department of Biology, University of Pisa, Pisa, Italy.

11:20 **Diatom blooms, chytrid epidemics and the evolutionary ecology of its interactions**
Ibelings, Bastiaan^{1,2}; De Bruin, Arnout²; Kagami, Maiko^{3,2}; Van Donk, Ellen².
1. Aquatic Ecology, Eawag, Kastanienbaum, Switzerland. 2. Foodweb Studies, NIOO, Nieuwersluis, Netherlands. 3. Environmental Science, Toho University, Funabashi, Japan.

11:50 **LUNCH BREAK**

12:00 – 1:30 *Journal of Phycology* Editorial Board Luncheon, **Barrington Room** (lower level)

12:00 – 1:30 *Journal of Eukaryotic Microbiology*, Editorial Board Luncheon, **Remingtons Restaurant**

12:00 – 1:00 Ticketed (pre-paid) lunches, **Rotunda**

SALON III-V, Monday Afternoon:

Bold Award 1

Presiding: Milton Sommerfeld, Arizona State University

1:30 **The effects of *in situ* and mesocosm nutrient additions on the epiphytic algal community of *Vallisneria americana* from the lower St. Johns river, Florida**
Dunn, Angela¹; Dobberfuhl, Dean²; Casamatta, Dale¹.
1. Department of Biology, University of North Florida, Jacksonville, FL, USA. 2. St Johns River Water Management District, Palatka, FL, USA.

1:50 **You can't judge a book by looking at the cover: understanding biodiversity of the conjugating green algae**
Hall, John.
Cell Biology and Molecular Genetics, University of Maryland, College Park, MD, USA.

2:10 **Are *Antithamnion pectinatum*, *Centroceras clavulatum* and *Spyridia filamentosa* (Rhodophyta) cosmopolitan red algal species?**
Won, Boo¹; Cho, Tae²; Suzanne, Fredericq¹.
1. Biology, University of Louisiana at Lafayette, Lafayette, LA, USA. 2. Marine Life Science, Chosun University, Gwangju, South Korea.

2:30 **Molecular analysis on the mating type locus of *Gonium pectorale* (Volvocales, Chlorophyta)**
Hamaji, Takashi¹; Takahashi, Fumio²; Nishii, Ichiro³; Nozaki, Hisayoshi¹.
1. Department of Biological Science, Graduate School of Science, University of Tokyo, Bunkyo-ku, Japan. 2. Department of Biomolecular Sciences, Graduate School of Life Sciences, Tohoku University, Sendai-shi, Japan. 3. Frontier Research System, RIKEN, Wako-shi, Japan.

- 2:50 **A phylogenetic and morphometric study of the freshwater green alga *Pediastrum duplex* (Sphaeropleales, Chlorophyceae)**
McManus, Hilary.
Ecology and Evolutionary Biology, University of Connecticut, Storrs, CT, USA.
- 3:10 **Utilizing an integrative taxonomic approach of molecular and morphological characters to delimit species in the red algal family Kallymeniaceae (Rhodophyta)**
Clarkston, Bridgette; Saunders, Gary.
University of New Brunswick, Fredericton, NB, Canada.
- 3:30 **BREAK**
- 3:50 **Gene expression in phlorotannin-rich tissue from the brown alga *Fucus vesiculosus***
Pelletreau, Karen; Coyne, Kathryn; Targett, Nancy.
College of Marine and Earth Studies, University of Delaware, Lewes, DE, USA.
- 4:10 **Ecology of the fish associated dinoflagellate *Crepidodinium cyprinodontum***
Cooney, Sean K.^{1,3}; Stoecker, Diane²; Coats, D. Wayne³.
1. Marine, Estuarine, and Environmental Sciences Program, University of Maryland, College Park, MD, USA. 2. University of Maryland, Center for Environmental Science, Cambridge, MD, USA. 3. Smithsonian Environmental Research Center, Edgewater, MD, USA.
- 4:30 **Significance of diatom EPS in food webs of Colne estuary biofilms: tracking ¹³C through lipids and polysaccharides tells the story**
Bellinger, Brent^{4,1}; Underwood, Graham²; Ziegler, Susan³; Gretz, Michael⁴.
1. Soil and Water Science Department, University of Florida, West Palm Beach, FL, USA. 2. Department of Biological Sciences, University of Essex, Colchester, United Kingdom. 3. Department of Earth Sciences, Memorial University of Newfoundland, St. John's, NF, Canada. 4. Department of Biological Sciences, Michigan Technological University, Houghton, MI, USA.
- 4:50 **Taxonomic reassessment of the *Caloglossa leprieurii*-complex (Delesseriaceae, Rhodophyta), and experimental elucidation of the function of pit connections**
Krayesky, David¹; Norris, James²; West, John³; Fredericq, Suzanne¹.
1. Dept. of Biology, University of Louisiana, Lafayette, LA, USA. 2. Dept. of Botany, Smithsonian Institution, Washington, DC, USA. 3. Dept. of Botany, University of Melbourne, Parkville, VIC, Australia.
- 5:10 **Insights into the growth and feeding of a temperate isolate of *Mesodinium rubrum***
Smith, Morten; Hansen, Per Juel.
Marine Biological Laboratory, University of Copenhagen, Helsingør, Denmark.

Salon I-II, Monday Afternoon:

ISOP Contributed Papers: Ecology|Symbiosis

Presiding: Andrea Habura, Wadsworth Center and Gabriela W. Smalley, Rider University

- 1:50 **Do planktonic euryhaline protists exist?**
Lovejoy, Connie^{1,2}.
1. Biology, Laval University, Quebec, QC, Canada. 2. Québec Ocean, Laval University, Québec, QC, Canada.
- 2:10 ***Acanthamoeba* distribution in the Chester River on the eastern shore of Maryland**
Munson, Donald; Bell, Christina.
Environmental Studies, Washington College, Chestertown, MD, USA.
- 2:30 **Amoebae from saline environments harbor *Legionella* species**
Gast, Rebecca¹; Moran, Dawn¹; Dennett, Mark¹; Rocca, Jennifer²; Amaral-Zettler, Linda².
1. Biology, Woods Hole Oceanographic Institution, Woods Hole, MA, USA. 2. Marine Biological Laboratory, Woods Hole, MA, USA.
- 2:50 **The non-pathogenic X-bacteria (*Candidatus legionella jeonii*) in symbiosis in *Amoeba proteus* have variations in pathogenicity genes in a comparative genomic analysis**
Kim, Hye Yeon; Lee, Kyung Min; Ahn, Tae In.
Biological Sciences, Seoul National University, Seoul, South Korea.
- 3:10 **A recent plastid establishment in the thecate amoeba *Paulinella chromatophora***
Yoon, Hwan Su¹; Reyes-Prieto, Adrian²; Bhattacharya, Debashish².
1. Bigelow Laboratory for Ocean Sciences, West Boothbay Harbor, ME, USA. 2. Department of Biological Sciences and Roy J. Carver Center for Comparative Genomics, University of Iowa, Iowa City, IA, USA.
- 3:30 **BREAK**
- 3:50 **Symbiont heterogeneity in reef foraminifera**
Fay, Scott.
Integrative Biology, UC Berkeley, Berkeley, CA, USA.
- 4:10 **Plastid retention and functionality in the dinoflagellates *Dinophysis acuminata* and *Dinophysis caudata***
Park, Myung¹; Park, Jong¹; Kim, Miran¹; Yih, Wonho².
1. LOHABE, Department of Oceanography, Chonnam National University, Gwangju, South Korea. 2. Department of Oceanography, Kunsan National University, Kunsan, South Korea.
- 4:30 **A day in the life of the zooxanthellate ciliate *Maristentor dinoferus*: a dispersal rhythm and behavioral defenses against grazing fish**
Lobban, Christopher S.; Schefter, Maria.
University of Guam, Mangilao, GU, USA.

- 4:50 **Phototrophic *Myrionecta rubra* is a remarkable “marine linking ciliate”**
Kim, Hyung Seop¹; Myung, Geumog¹; Seong, Kyeong Ah¹; Park, Jong Soo²; Jeong, Hae Jin²; Cho, Byung Cheol²; Yih, Wonho¹.
1. Dept. Oceanography, Kunsan National University, Kunsan, South Korea. 2. School of Earth and Environmental Sciences, Seoul National University, Seoul, South Korea.
- 5:10 **EOL – building bridges between barcodes and traditional information**
Patterson, David.
MBL, Woods Hole, MA, USA.

Grand Foyer West, Monday Evening:

7:00 to 9:30 **JOINT PSA-ISOP AUCTION AND MIXER**

TUESDAY, 7 AUGUST

6:50 – 7:50 Continental Breakfast in Rotunda

Salon III-V, Tuesday Morning:

PSA Special Session: Physiological and Structural Advantages of Chloroplast Evolution

Introducing and Presiding: John La Claire, University of Texas

- 8:00 **PLENARY LECTURE: Chloroplast evolution: past and present**
Gantt, Elizabeth.
Cell Biology and Molecular Genetics, University of Maryland, College Park, MD, USA.
- 8:50 **The cyanelle of *Cyanophora paradoxa*: missing link of plastid evolution**
Loeffelhardt, Wolfgang.
Department of Biochemistry, Max F. Perutz Laboratories, University of Vienna, Vienna, Austria.
- 9:30 **Plastid kleptoplasty: one first step toward endosymbiosis**
Worful, Jared¹; Kannan, Krishna¹; Lee, Jungho²; Soule, Kara¹; Manhart, James³; Rumpho, Mary¹.
1. Biochemistry, University of Maine, Orono, ME, USA. 2. Green Plant Institute, Seoul National University, Suwon, South Korea. 3. Biology, Texas A&M University, College Station, TX, USA. 4. Biology, University of Maine, Orono, ME, USA.
- 10:00 **BREAK**

Bold Award2

Presiding: Milton Sommerfeld, Arizona State University

- 10:30 **Across space and over time: processes that influence population genetic structure of *Fucus vesiculosus* l. In the northwestern Atlantic**

Muhlin, Jessica; Brawley, Susan.

School of Marine Sciences, University of Maine, Orono, ME, USA.

- 10:50 **Axenic cultivation of the heterotrophic dinoflagellate *Pfiesteria shumwayae* and observations on feeding behavior**

Skelton, Hayley¹; Burkholder, JoAnn¹; Parrow, Matthew².

1. Center for Applied Aquatic Ecology, North Carolina State University, Raleigh, NC, USA.

2. Biology, University of North Carolina at Charlotte, Charlotte, NC, USA.

- 11:10 **Understanding dinoflagellate genome: insights from genomic data and PCNA characteristics**

Hou, Yubo; Lin, Senjie.

Marine Sciences, University of Connecticut, Groton, CT, USA.

- 11:30 **Effects of nutrient limitation and salinity stress on growth and photosynthesis in the green alga *Picochlorum oklahomensis***

Annan, J. Nana; Henley, William.

Botany Department, Oklahoma State University, Stillwater, OK, USA.

- 11:50 **Morphometric, reproductive and molecular characterization of *Pseudo-nitzschia delicatissima* (Cleve) Heiden**

Reid, Charlotte¹; Kaczmarska, Irena¹; Martin, Jennifer².

1. Biology, Mount Allison University, Sackville, NB, Canada. 2. Department of Fisheries and Oceans, St. Andrews Biological Station, St. Andrews, NB, Canada.

- 12:10 **LUNCH BREAK**

12:00 – 1:00 Ticketed (pre-paid) lunches, Rotunda

12:00 – 2:00 PSA Student-Postdoc luncheon, Atrium

Bristol A-B, Tuesday Morning:

ISOP Special Session: Drug Targets In Parasitic Protists

Presiding: Nigel Yarlett, Haskins Laboratories, Pace University

- 8:00 **Opening Remarks**

- 8:10 **Giardan: structure, synthesis, regulation and inhibition**

Jarroll, Edward^{1,2}.

1. Biology, Northeastern University, Nahant, USA. 2. Biology, Northeastern University, Boston, MA, USA.

- 8:30 **The ‘hydrogenosome’ at thirty-four years of age and its role in the activity of metronidazole**
Lindmark, Donald.
Biology, Cleveland State University, Cleveland, OH, USA.
- 8:50 **ATP binding cassette (ABC) transporters and drug efficacy in *Cyptosporidium parvum***
Mead, Jan^{1,2}; Benitez, Alvaro²; McNair, Nina¹.
1. Atlanta VA Medical Center, Decatur, GA, USA. 2. Pediatrics, Emory University, Atlanta, GA, USA.
- 9:10 **Novel compartmentalization of *Cryptosporidium parvum* pyruvate:NADP⁺ oxidoreductase within the crystalloid body**
Ctrnacta, Vlasta²; Stejskal, Frantisek²; Buttle, Karolyn³; Mannella, Carmen³; Hsieh, Chong-ere³; Marko, Mike³; Wynalek, Jessica^{4,1}; Keithly, Janet¹.
1. Infectious Disease, Wadsworth Center, New York State Department of Health, Albany, NY, USA. 2. Tropical Medicine and Parasitology, Charles University, Prague, Czech Republic. 3. Resource for the Visualization of Biological Complexity, Wadsworth Center, New York State Department of Health, Albany, NY, USA. 4. APHL/CDC/EID Fellow, Wadsworth Center, New York State Department of Health, Albany, NY, USA.
- 9:30 **Effects of ion-channel blockers on *Plasmodium falciparum* viability and their potential use as anti-malarial targets.**
Martiney, James.
Biology and Health Sciences, Pace University, New York, NY, USA.
- 9:50 **BREAK**
- 10:20 **Title TBA**
Perez, Oscar
Temple University School of Medicine, Philadelphia, PA, USA.
- ISOP Contributed Papers: Cell Biology\Physiology\Behavior**
Presiding: Aaron Bell, Albert Einstein College of Medicine of Yeshiva University
- 10:40 **Action of dinitroaniline herbicides in the protozoan parasite *Toxoplasma gondii***
Ayana, Marge; Bertozzi, Danelle; Heino, Aino; Judkins, Myeika; Keogan, Shawn; Story, Elizabeth; Fichera, Maria.
Biology, Eastern University, St. Davids, PA, USA.
- 11:00 **Friendly iron and adversary zinc modulate trophozoite growth of *Entamoeba histolytica***
Espinosa, Avelina; Arnold, Shannon.
Biology, Roger Williams University, Bristol, RI, USA.
- 11:20 **Motile behavior of *Trichomonas*: lack of response to oxygen gradients, and fractal dispersion**
Levandowsky, Michael; Yarlett, Nigel; Yu, Jieying.
Haskins Labs, Pace University, New York, NY, USA.

11:40 **Identification and characterization of arf guanine nucleotide exchange factors of the Sec7 family in *Tetrahymena thermophila***
Bell, Aaron; Guerra, Charles; Satir, Peter.
ASB, AECOM, Bronx, NY, USA.

12:00 **LUNCH BREAK**

12:00 – 1:00 Ticketed (pre-paid) lunches, Rotunda

Salon III-V, Tuesday Afternoon:

ISOP Hutner Lecture

Introducing and Presiding: John C. Clamp, North Carolina Central University

1:30 **Interactions among marine protists: importance of mixotrophy, pH and toxins**
Hansen, Per.
Marine Biological Laboratory, University of Copenhagen, Helsingør, Denmark.

ISOP Symposium: Biogeochemical Cycling: The Protist Connection

Presiding: Joan M. Bernhard, Woods Hole Oceanographic Institution and Samuel S. Bowser, Wadsworth Center

2:30 **“Extremophile” foraminifera and H, N, C, and S cycling**
Bernhard, Joan.
Geology & Geophysics, Woods Hole Oceanographic Institution, Woods Hole, USA.

3:00 **Methane from protists and methane for protists: their contribution to methane production, and their effect upon methane oxidation**
Frenzel, Peter.
MPI for Terrestrial Microbiology, Marburg, Germany.

3:30 **BREAK**

3:50 **Carbonate production in the world's ocean: the role of foraminifera**
Langer, Martin.
Earth Sciences, Institute of Paleontology, Bonn, Germany.

4:20 **The role of protozoa in ocean iron biogeochemistry**
Twining, Benjamin.
University of South Carolina, Columbia, SC, USA.

Salon I-II, Tuesday Afternoon:

PSA Contributed Papers: Physiology and Biochemistry

Presiding: T.J. Evens, USDA-ARS

- 2:30 **Dinitrogen fixation by a marine phytoplankton ecosystem**
Li, Zhongkui¹; Kim, Kyoung-Rae²; Brand, Jerry¹.
1. MCD-Biology, Univ. of Texas at Austin, Austin, TX, USA. 2. College of Pharmacy, Sungkyunkwan University, Suwon, South Korea.
- 2:50 **Steps towards unraveling the regulation of nitrogen assimilation in the marine diatom *Thalassiosira pseudonana*: oscillations in mRNA levels of five key nitrogen-assimilating enzymes**
Brown, Kathryn; Twing, Katrina; Robertson, Deborah.
Clark University, Worcester, MA, USA.
- 3:10 **Nitrate uptake rate in *Porphyra* species from different tide levels: responses to desiccation stress**
Kim, Jang^{1,3}; Kraemer, George²; Yarish, Charles¹.
1. Department of Ecology & Evolutionary Biology, University of Connecticut, Stamford, CT, USA. 2. Departments of Biology & Environmental Studies, Purchase College, State University of New York, Purchase, NY, USA. 3. Department of Ecology & Evolutionary Biology, University of Connecticut, Groton, CT, USA.
- 3:30 ***Didymosphenia geminata* as a nuisance diatom: runaway stalk production results in mats with significant environmental impact**
Gretz, Michael¹; Riccio, Michelle¹; Kiemle, Sarah¹; Domozych, David²; Spaulding, Sarah³.
1. Biotechnology Research Center, Michigan Technological University, Houghton, MI, USA. 2. Department of Biology, Skidmore College, Saratoga Springs, NY, USA. 3. U.S. Geological Survey / EPA Region VII, Denver, CO, USA.
- 3:50 **Comparative effects of herbicides on photosynthesis and growth of the tropical microalga *Nephroselmis pyriformis***
Magnusson, Marie^{2,3}; Heimann, Kirsten¹; Negri, Andrew⁴; Ridd, Michael³.
1. School of Marine and Tropical Biology, James Cook University, Townsville, QLD, Australia. 2. AIMS@JCU, Australian Institute of Marine Science, Townsville, QLD, Australia. 3. School of Pharmacy and Molecular Sciences, James Cook University, Townsville, QLD, Australia. 4. Australian Institute of Marine Science, Townsville, QLD, Australia.
- 4:10 **BREAK**
- 4:30 ***PSA Business Meeting in Bristol A-B***

Bristol A-B, Tuesday Afternoon:

PSA Contributed Papers: Ecology and Population Biology 1

Presiding: Carol Thornber, University of Rhode Island

- 2:30 **Phylogeography of *Asparagopsis taxiformis* (Bonnemaisoniales, Rhodophyta) in the Hawaiian Islands: two mtDNA markers support three separate introductions**
Sherwood, Alison.
Botany, University of Hawaii, Honolulu, HI, USA.
- 2:50 **Submersed plants of the Indian River Lagoon: a floristic inventory and field guide**
Littler, Diane^{1,2}; Littler, Mark²; Hanisak, M.Dennis¹.
1. Harbor Branch Oceanographic Institution, Fort Pierce, FL, USA. 2. Department of Botany, Smithsonian Institution, Washington, DC, USA.
- 3:10 **European influences on the North American shore**
Brawley, Susan¹; Coyer, James²; Hoarau, Galice²; Johnson, Ladd³; Stam, Wytze²; Blakeslee, April⁴; Cunningham, Cliff⁵; Olsen, Jeanine².
1. School of Marine Sciences, University of Maine, Orono, ME, USA. 2. MarBEE, The Biological Centre, University of Groningen, 9750 AA Haren, Netherlands. 3. Département de biologie, Université Laval, Québec, QC, Canada. 4. Department of Biology, University of New Hampshire, Durham, NH, USA. 5. Biology Department, Duke University, Durham, NC, USA.
- 3:30 **Variation in wave forces along vertical transects in the wave-swept rocky intertidal zone as a potential driver for macroalgal distribution**
Boller, Michael; Finkler, Tad.
Hopkins Marine Station of Stanford University, Pacific Grove, CA, USA.
- 3:50 **The progression of the invasive red alga *Grateloupia turuturu* Yamada along the Connecticut coastline in Long Island Sound, USA**
Gladych, Rebecca¹; Kraemer, George²; Zhang, Huan¹; Lin, Senjie¹; Yarish, Charles^{1,3}.
1. Department of Marine Sciences, University of Connecticut, Groton, CT, USA. 2. Department of Environmental Studies, Purchase College, State University of New York, Purchase, NY, USA. 3. Department of Ecology and Evolutionary Biology, University of Connecticut, Stamford, CT, USA.
- 4:10 **BREAK**
- 4:30 ***PSA Business Meeting***

Rotunda, Tuesday Evening:

8:00 – 10:00 PM PSA Student/Postdoc mixer

WEDNESDAY, 8 AUGUST

6:50 – 7:50 Continental Breakfast in Rotunda

Salon III-V, Wednesday Morning:

PSA Special Session: Phylogenetics, Systematics, and Biogeography of Macroalgae

Introducing and Presiding: Suzanne Fredericq, University of Louisiana at Lafayette

8:00 PLENARY LECTURE: Phylogeny, systematics and biogeography of the red algae
Hommersand, Max.

Department of Biology, University of North Carolina, Chapel Hill, NC, USA.

9:00 Evolutionary biogeography of siphonous green algae

Verbruggen, Heroen; Pauly, Klaas.

Ghent University, Ghent, Belgium.

9:30 Our current understanding of brown algal evolution

Draisma, Stefano.

PCNE, Nationaal Herbarium Nederland - Universiteit Leiden branch, Leiden, Netherlands.

10:00 BREAK

PSA Contributed Papers: Phylogenetics and Taxonomy 1

Presiding: Curt Pueschel, SUNY-Binghamton

10:30 'Super' plant kingdom reinstated: nonmonophyly of primary photosynthetic eukaryotes as deduced from slowly evolving nuclear genes

Nozaki, Hisayoshi¹; Iseki, Mineo²; Hasegawa, Masami³; Misawa, Kazuharu¹; Nakada, Takashi¹; Sasaki, Narie⁴; Watanabe, Masakatsu⁵.

1. Department of Biological Sciences, Graduate School of Science, University of Tokyo, Bunkyo-ku, Japan. 2. Hayama Center for Advanced Studies, Graduate University for Advanced Studies, Hayama, Japan. 3. School of Life Sciences, Fudan University, Shanghai, China. 4. Division of Biological Science, Graduate School of Science, Nagoya University, Nagoya-shi, Japan. 5. Department of Evolutionary Studies of Biosystems, School of Advanced Sciences, Graduate University for Advanced Studies, Hayama, Japan.

10:50 The red algal order Nemastomatales: new insights in the life history, morphology, phylogeny and biogeography of pertinent taxa

Gabriel, Daniela^{1,2}; Parente, Manuela¹; Neto, Ana¹; Fredericq, Suzanne².

1. Department of Biology, University of the Azores, Ponta Delgada, Portugal. 2. Department of Biology, University of Louisiana at Lafayette, Lafayette, LA, USA.

11:10 Comparison of three organelle markers for phylogeographic inference in *Batrachospermum helminthosum* (Batrachospermales, Rhodophyta) from North America

House, Denise¹; Sherwood, Alison²; Vis, Morgan¹.

1. Environmental & Plant Biology, Ohio University, Athens, OH, USA. 2. Department of Botany, University of Hawaii, Honolulu, HI, USA.

11:30 **Phylogeographic relationships among *Batrachospermum arcuatum* (Rhodophyta) collections throughout its distribution**

Vis, Morgan¹; Chiasson, Wayne¹; Stancheva, Rosalina²; Chou, Jui-Yu³.

1. *Env. & Plant Biology, Ohio University, Athens, OH, USA.* 2. *Department of Botany, Sofia University, Sofia, Bulgaria.* 3. *Faculty of Life Sciences, National Yang Ming University, Taipei City, Taiwan.*

11:50 **LUNCH BREAK**

12:00 – 1:00 **Ticketed (pre-paid) lunches, Rotunda**

Salon I-II, Wednesday Morning:

PSA Contributed Papers: Cellular and Molecular Biology 1

Presiding: Kathryn Coyne, University of Delaware

(note: one talk canceled just before program went to printer)

10:30 **The dinoflagellate genome: insights from full-length cDNAs for *Karlodinium* and *Amphidinium***

Lin, Senjie¹; Zhang, Huan¹; Place, Allen²; Adolf, Jason²; Gaasterland, Terry³; Rogers, Yu-Hui⁴; Gill, John⁴; Tran, Bao⁴.

1. *Marine Sciences, University of Connecticut, Groton, CT, USA.* 2. *Center of Marine Biotechnology, University of Maryland Biotechnology Institute, Baltimore, MD, USA.* 3. *Scripps Genome Center, Scripps Institution of Oceanography, La Jolla, CA, USA.* 4. *J. Craig Venter Institute, Rockville, MD, USA.*

10:40 **From stop to start: comparing expressed and genomic versions of genes in the dinoflagellate *Amphidinium carterae***

Bachvaroff, Tsvetan^{2,1}; Place, Allen².

1. *Smithsonian Environmental Research Center, Edgewater, MD, USA.* 2. *Center of Marine Biotechnology, University of Maryland Biotechnology Institute, Baltimore, MD, USA.*

11:10 **Search for growth-related genes in *Alexandrium fundyense* through cDNA microarray analysis**

Miranda, Lilibeth; Lin, Senjie.

Department of Marine Sciences, University of Connecticut, Groton, CT, USA.

11:30 **LUNCH BREAK**

12:00 – 1:00 **Ticketed (pre-paid) lunches, Rotunda**

Bristol A-B, Wednesday Morning:

ISOP Special Session: Protists and the Molecular and Informatics Revolution: from Species Pages to Barcodes

Presiding: Linda Amaral-Zettler, Marine Biological Laboratory, Woods Hole and Robert Andersen, CCMP, Bigelow Laboratory for Ocean Sciences

8:10 Opening Remarks

Invited Presentations

8:20 The international census of marine microbes and marine protists

Amaral-Zettler, Linda.

Marine Biological Laboratory, Woods Hole, MA, USA.

8:40 The barcode of life initiative

Hajibabaei, Mehrdad.

Canadian Centre for DNA Barcoding, Guelph, ON, Canada.

9:00 Capturing biological data and expertise with XML

Habura, Andrea^{1,2}.

1. Division of Molecular Medicine, Wadsworth Center, NYSDOH, Albany, NY, USA. 2. Department of Biomedical Sciences, University at Albany, Albany, NY, USA.

9:20 Protistan culture collections: their current status and their participation in barcoding initiatives

Andersen, Robert.

CCMP, Bigelow Laboratory for Ocean Sciences, West Boothbay Harbor, ME, USA.

9:40 Marine macroalgae as an exemplar of DNA barcoding among protistan lineages challenges widely held perspectives in biodiversity and biogeography

Saunders, Gary.

Biology, University of New Brunswick, Fredericton, NB, Canada.

10:00 BREAK

10:30 Comparative protistan biogeography

Caron, David.

Biological Sciences, University of Southern California, Los Angeles, CA, USA.

10:50 The eukaryotic tree of life assessed through increased taxon and gene sampling

Katz, Laura^{1,2}.

1. Biological Sciences, Smith College, Northampton, MA, USA. 2. Organismic and Evolutionary Biology, UMass-Amherst, Amherst, MA, USA.

Contributed Presentations

- 11:10 **Testing the effectiveness of cox-1 barcoding as a taxonomic tool to identify *Tetrahymena* species and to elucidate their evolutionary history**
Kher, Chandni; Lynn, Denis.
Integrative Biology, University of Guelph, Guelph, ON, Canada.
- 11:30 **Barcoding ciliates – a phylum with highly divergent COI sequences**
Strueder-Kypke, Michaela; Lynn, Denis.
Integrative Biology, University of Guelph, Guelph, ON, Canada.
- 11:50 **LUNCH BREAK**
- 12:00 – 1:00 Ticketed (pre-paid) lunches, Rotunda**
- 12:30 – 2:00 ***ISOP Business Meeting and Luncheon* – Barrington Room (lower level)**

Salon III-V, Wednesday Afternoon:

PSA Contributed Papers: Phylogenetics and Taxonomy 2

Presiding: Juan Lopez-Bautista, University of Alabama

- 1:50 **Linking the present and the past using Chrysophyte DNA sequences – new perspectives for cyst-based reconstructions**
Kamenik, Christian¹; Andersen, Robert².
1. Institute of Geography, University of Bern, Bern, Switzerland. 2. Bigelow Laboratory for Ocean Sciences, West Boothbay Harbor, ME, USA.
- 2:10 **Enigmatic reproductive structures in *Platysiphon verticellatus* wilce (1962): an Arctic annual endemic**
Wilce, Robert¹; Bradley, Peter².
1. Biology, Univ. of Massachusetts, Amherst, MA, USA. 2. Biology, Worcester State College, Worcester, MA, USA.
- 2:30 **Barcoding brown algae: the DNA barcode initiative is changing our view of the Phaeophyceae in Canada**
McDevit, Daniel; Saunders, Gary.
Biology, University of New Brunswick, Fredericton, NB, Canada.
- 2:50 **Phylogenetic reconstruction of the order Surirellales (Bacillariophyta) from morphological and DNA sequence data**
Ruck, Elizabeth; Theriot, Edward.
Integrative Biology, University of Texas at Austin, Austin, TX, USA.
- 3:10 **BREAK**

PSA Contributed Papers: Phylogenetics and Taxonomy 3

Presiding: Louise Lewis, University of Connecticut, Storrs

3:30 **Differential occurrence of land plant extracellular polysaccharides in the charophycean green algae and implications for plant evolution**

Domozych, David¹; Kiemle, Sarah²; Domozych, Catherine¹; Sørensen, Iben³; Willats, William³; Gretz, Michael².

1. Department of Biology and Skidmore Microscopy Imaging Center, Skidmore College, Saratoga Springs, NY, USA. 2. Department of Biological Sciences and Biotechnology Research Center, Michigan Technological University, Houghton, MI, USA. 3. Department of Molecular Biology, Copenhagen Biocentre, Copenhagen, Denmark.

3:50 **The green symbionts of *Anthopleura* form a distinct monophyletic taxon in Trebouxiophyceae (Chlorophyta)**

Letsch, Molly¹; Lewis, Louise¹; Muller-Parker, Gisèle².

1. Ecology and Evolutionary Biology, University of Connecticut, Storrs, CT, USA. 2. Biology, Western Washington University, Bellingham, WA, USA.

4:10 **Tropical subaerial microchlorophytes and the quest for land**

Lopez-Bautista, Juan.

Biological Sciences, The University of Alabama, Tuscaloosa, AL, USA.

4:30 **Diversity, evolution and ecology of the Trentepohliales (Ulvophyceae, Chlorophyta) in tropical regions**

Rindi, Fabio; Lopez-Bautista, Juan.

Biological Sciences, The University of Alabama, Tuscaloosa, AL, USA.

4:50 **The search for class and ordinal level synapomorphies in *Spongiochrysis hawaiiensis* (Ulvophyceae, Chlorophyta)**

Lam, Daryl; Rindi, Fabio; Lopez-Bautista, Juan.

Biological Sciences, University of Alabama, Tuscaloosa, AL, USA.

5:10 **Investigations on the subaerial Trentepohliales (Ulvophyceae, Chlorophyta) in the southeastern USA**

Noble, Sarah; López-Bautista, Juan.

The University of Alabama, Tuscaloosa, AL, USA.

Salon I-II, Wednesday Afternoon:

PSA Contributed Papers: Ecology and Population Biology 2

Presiding: Wayne Litaker, National Ocean Service, NOAA

1:30 **Towards an improved understanding of *Cyanidiales* ecology and their contribution to biogeochemical cycling in geothermal environments**

McDermott, Timothy¹; Castenholz, Richard².

1. Land Resources & Environmental Sciences and Thermal Biology Institute, Montana State University, Bozeman, MT, USA. 2. Center for Ecology and Evolutionary Biology, University of Oregon, Eugene, OR, USA.

- 1:50 **Prey-induced changes in swimming behavior of predatory dinoflagellates**
Sheng, Jian¹; Malkiel, Edwin¹; Katz, Joseph¹; Adolf, Jason²; Belas, Robert²; Place, Allen².
 1. *Dept. of Mechanical Engineering, The Johns Hopkins University, Baltimore, MD, USA.* 2. *Center of Marine Biotechnology, University of Maryland Biotechnology Institute, Baltimore, MD, USA.*
- 2:10 **Fine scale distribution and abundance of large and small phytoplankton in Monterey Bay, CA**
McFarland, Malcolm; Rines, Jan; Donaghay, Percy; Sullivan, James.
Graduate School of Oceanography, University of Rhode Island, Narragansett, RI, USA.
- 2:30 **Chrysophytes and diatoms from an Eocene Arctic lake: implications for biogeography, and evolutionary stasis**
Siver, Peter¹; Wolfe, Alex².
 1. *Botany, Connecticut College, New London, CT, USA.* 2. *Department of Earth and Atmospheric Sciences, University of Alberta, Edmonton Alberta, AB, Canada.*
- 2:50 **Great Lakes diatom tools: advantages over chemical measurements in paleolimnological and monitoring programs**
 Reavie, Euan.
Natural Resources Research Institute, University of Minnesota Duluth, Ely, MN, USA.

3:10 **BREAK**

PSA Contributed Papers: Ecology and Population Biology 3

Presiding: Peter Siver, Connecticut College

- 3:30 **Effects of *Karlodinium veneficum* on early life history stages of the eastern oyster**
Stoecker, Diane¹; Adolf, Jason²; Place, Allen²; Glibert, Patricia¹; Meritt, Donald¹.
 1. *UMCES, HPL, Cambridge, MD, USA.* 2. *UMBI, COMB, Baltimore, MD, USA.*
- 3:50 **Development of a toxic dinoflagellate (*Karlodinium veneficum*) bloom in a shallow, eutrophic, lagoonal estuary**
Litaker, Wayne¹; Hall, Nathan²; Fensin, Elizabeth³; Adolf, Jason⁴; Place, Allen⁴; Paerl, Hans².
 1. *National Ocean Service, NOAA, Beaufort, NC, USA.* 2. *Institute of Marine Sciences, University of North Carolina at Chapel Hill, Morehead City, NC, USA.* 3. *Division of Water Quality, North Carolina Department of Environment and Natural Resources, Raleigh, NC, USA.* 4. *Center of Marine Biotechnology, University of Maryland Biotechnology Institute, Baltimore, MD, USA.*
- 4:10 **Grazing on the estuarine bloom forming species *Karlodinium veneficum* deterred by production of karlotoxins**
Tester, Patricia¹; Waggett, Rebecca¹; Place, Allen².
 1. *National Ocean Service, NOAA, Beaufort, NC, USA.* 2. *Center of Marine Biotechnology, University of Maryland Biotechnology Institute, Baltimore, MD, USA.*

- 4:30 **Strain variability in *Karlodinium veneticum* (Dinophyceae)**
Place, Allen¹; Adolf, Jason¹; Bachvaroff, Tsvetan¹; Zhang, Huan²; Lin, Senjie².
 1. Center of Marine Biotechnology, University of Maryland Biotechnology Institute, Baltimore, MD, USA. 2. Department of Marine Sciences, University of Connecticut, Groton, CT, USA.
- 4:50 **Cryptophytes drive blooms of mixotrophic harmful algae: a testable hypothesis based on *Karlodinium veneticum* in Chesapeake Bay**
Adolf, Jason; Bachvaroff, Tsvetan; Place, Allen.
 COMB, UMBI, Baltimore, MD, USA.
- 5:10 ***Prymnesium* in Florida: an emerging harmful algae problem**
Wolny, Jennifer¹; Landsberg, Jan²; Tomas, Carmelo³.
 1. Fish and Wildlife Research Institute, Florida Institute of Oceanography, St. Petersburg, FL, USA. 2. Fish and Wildlife Research Institute, Florida Fish and Wildlife Conservation Commission, St. Petersburg, FL, USA. 3. Center for Marine Science, University of North Carolina - Wilmington, Wilmington, NC, USA.

Plaza Ballroom and Foyer, Wednesday Evening:

Joint PSA-ISOP Poster Session and Mixer: 7:00 to 9:30 PM

Presenting authors are encouraged to stand with their posters throughout the evening.

Presenting authors of ODD numbered posters are requested to stand with their posters at least between 7:00 and 7:45.

Presenting authors of EVEN numbered posters are requested to stand with their posters at least between 7:45 and 8:30.

Posters can be put up on Monday and can be left up until Thursday morning. Authors are encouraged to have their posters on display for as long as possible.

- P1 ***Trachelomonas* in Iowa wetland mitigation sites**
Main, Stephen; Shellabarger, Rachel.
 Biology, Wartburg College, Waverly, IA, USA.
- P2 **Role of algae in assessing aquatic ecological conditions of wadeable streams for USGS NAWQA surface water status and trends**
Hambrook Berkman, Julie¹; Frey, Jeffrey²; Sullivan, Daniel³; Carpenter, Kurt⁴; Mabe, Jeffrey⁵.
 1. US Geological Survey, Columbus, OH, USA. 2. US Geological Survey, Indianapolis, IN, USA. 3. US Geological Survey, Middleton, WI, USA. 4. US Geological Survey, Portland, OR, USA. 5. US Geological Survey, Austin, TX, USA.
- P3 **Characterization of a rocky intertidal shoreline within Acadia National Park: impact of short-term trampling and implications for management**
Olson, David; Brawley, Susan; Wilson, James.
 School of Marine Sciences, University of Maine, Orono, ME, USA.

- P4 **The effects of water phosphate concentrations on the distribution and degree of calcification of two calcareous green macroalgae**
Glenn, Kyle; Bedinger, Laura; Bell, Susan; Dawes, Clinton.
Integrative Biology, University of South Florida, Tampa, FL, USA.
- P5 **A report on postage stamps depicting algae**
 Wynne, Michael.
Ecology & Evolutionary Biology, University of Michigan, Ann Arbor, MI, USA.
- P6 **Desmid-rich biofilms and heterotrophic protist assemblages of Adirondack wetlands**
Domozych, David¹; Anderson, O. Roger²; Domozych, Catherine¹.
 1. *Biology, Skidmore College, Saratoga Springs, NY, USA.* 2. *Biology, Lamont-Doherty Earth Observatory of Columbia University, Palisades, NY, USA.*
- P7 **Effects of light intensity and prey concentration on growth, grazing, and survival of the mixotrophic dinoflagellate *Dinophysis acuminata***
Kim, Sunju¹; Kang, Yi Gu¹; Kim, Hyung Seop¹; Yih, Wonho¹; Park, Myung Gil².
 1. *Oceanography, Kunsan Nat'l Univ., Kunsan, South Korea.* 2. *Oceanography, Chonnam Nat'l Univ., Gwangju, South Korea.*
- P8 **Comparison of green algal bloom intensity and related water quality parameters at paired “bloom” and “non-bloom” sites**
Peter, Lukas¹; Nelson, Timothy¹; Van Alstyne, Kathryn¹; Ronhovde, Erica¹; Gifford, Suzy²; Cataldo, Marianne²; Nicely, Alexandra²; Puglisi, Melani².
 1. *Biology, Seattle Pacific University, Seattle, WA, USA.* 2. *Shannon Point Marine Center, Western Washington University, Anacortes, WA, USA.*
- P9 **Underwater video analysis allows for the mapping of green algal blooms throughout the inland marine waters of Washington state**
Guerra, Carmen; Nelson, Timothy; Ronhovde, Erica; Peter, Lukas.
Biology, Seattle Pacific University, Seattle, WA, USA.
- P10 **Recruitment of *Padina australis* Hauck (Phaeophyta), at Sirinart National Park and Tang Khen Bay, Phuket Province, Thailand**
Wichachucherd, Bongkot¹; Liddle, Larry²; Prathep, Anchana¹.
 1. *Seaweed and Seagrass Research Unit, Prince of Songkla University, Hat Yai, Thailand.* 2. *Marine Science, Long Island University, Southampton, NY, USA.*
- P11 **Influence of physical trampling disturbance on desert soil food webs associated with biological soil crusts**
Darby, Brian¹; Housman, David²; Johnson, Shannon³; Neher, Deborah¹; Kuske, Cheryl³; Belnap, Jayne².
 1. *Plant and Soil Science, University of Vermont, Burlington, VT, USA.* 2. *Canyonlands Research Station, US Geological Survey, Moab, UT, USA.* 3. *Environmental Molecular Biology, Biosciences Division, Los Alamos National Laboratory, Los Alamos, NM, USA.*

- P12 **Molecular methods for investigating natural diatom communities: Delaware inland bays and beyond**
Handy, Sara^{1,2}; Salvitti, Lauren²; Coyne, Kathryn².
 1. *Cell Biology and Molecular Genetics, University of Maryland, Silver Spring, MD, USA.* 2. *College of Marine and Earth Studies, University of Delaware, Lewes, DE, USA.*
- P13 **Cyanobacteria in eutrophic, turbid impoundments of the North Carolina piedmont**
Burkholder, JoAnn¹; Allen, Elle¹; Kinder, Carol¹; Touchette, Brant²; James, Jennifer¹.
 1. *Center for Applied Aquatic Ecology, North Carolina State University, Raleigh, NC, USA.* 2. *Center for Environmental Studies, Elon University, Elon, NC, USA.*
- P14 **First records of protostelids (Eumycetozoa) from the Antarctic Peninsula**
Shadwick, John¹; Timling, Ina²; Stephenson, Steven¹; Spiegel, Frederick¹.
 1. *Department of Biological Sciences, University of Arkansas, Fayetteville, AR, USA.* 2. *Department of Biology and Wildlife, University of Alaska, Fairbanks, AK, USA.*
- P15 **Using gut content analysis to assess macroalgae importance as a food source for the amphipod community endemic to western Antarctic Peninsula**
Aumack, Craig¹; Amsler, Charles¹; McClintock, Jim¹; Baker, Bill².
 1. *Department of Biology, University of Alabama Birmingham, Birmingham, AL, USA.* 2. *Department of Chemistry, University of South Florida, Tampa, FL, USA.*
- P16 **The ability of a single-cell alkaline phosphatase assay (ELF-97) to indicate phosphate stress and cellular P content in two strains of the mixotrophic dinoflagellate, *Prorocentrum minimum***
 Sliko, Carey¹; Golinski, Alison^{1,2}; Zabrocky, Susan¹; Smalley, Gabriela¹.
 1. *Geological, Environmental, and Marine Sciences, Rider University, Lawrenceville, NJ, USA.* 2. *Animal Science, Rutgers University, New Brunswick, NJ, USA.*
- P17 **Regional distribution of notodendroid foraminifera in McMurdo Sound, Antarctica: is *Notodendrodes antarctikos* an endangered protist?**
Bowser, Samuel^{1,2}; Habura, Andrea^{1,2}; Alexander, Stephen¹; Hanes, Steven^{1,2}; Pawlowski, Jan³.
 1. *Division of Molecular Medicine, Wadsworth center, NYSDOH, Albany, NY, USA.* 2. *Department of Biomedical Sciences, University at Albany, Albany, NY, USA.* 3. *Department of Zoology and Animal Biology, University of Geneva, Geneva, Switzerland.*
- P18 **Chemical defenses against diatom fouling in Antarctic macroalgae: insights from bioassay guided fractionation**
Sevak, Hamel¹; Amsler, Margaret¹; Maschek, J. Alan²; Amsler, Charles¹; McClintock, James¹; Baker, Bill².
 1. *Department of Biology, University of Alabama at Birmingham, Birmingham, AL, USA.* 2. *Chemistry, USF, Tampa, FL, USA.*
- P19 ***Pentapharsodinium tyrrhenicum* is a parasitic dinoflagellate of the ctenophore *Mnemiopsis leidyi***
Smith, Khristian¹; Dodson, Matthew¹; Santos, Scott¹; Gast, Rebecca²; Rogerson, Andrew³; Sullivan, Barbara⁴; Moss, Anthony^{1,2}.
 1. *Biological Sciences, Auburn University, Auburn, AL, USA.* 2. *Biology, Woods Hole Oceanographic, Woods Hole, MA, USA.* 3. *College of Sciences, Marshall University, Huntington, WV, USA.* 4. *Oceanography, University of Rhode Island, Narragansett, RI, USA.*

- P20 **Macroalgal colonization: recruitment of a new rocky habitat along the south Texas coast**
Fikes, Ryan; Lehman, Roy.
Department of Life Sciences, Center for Coastal Studies, Texas A&M University-Corpus Christi, Corpus Christi, TX, USA.
- P21 **Protistan distribution in relation to spatial variations of extreme geochemical parameters in the Rio Tinto, Spain**
Zettler, Erik^{1,2}; Amils, Ricardo^{1,3}; Theroux, Susanna⁴; Palacios, Carmen⁵; Sogin, Mitchell⁴; Amaral-Zettler, Linda⁴.
1. Centro de Biología Molecular, U. Autónoma de Madrid, Madrid, Spain. 2. Sea Education Association, Woods Hole, MA, USA. 3. Centro de Astrobiología, INTA-CSIC, Torrejón de Ardoz, Spain. 4. The Josephine Bay Paul Center for Comparative Molecular Biology and Evolution, Marine Biological Laboratory, Woods Hole, MA, USA. 5. Laboratoire microbiologie, Observatoire Oceanographique Banyuls, Banyuls Sur Mer, France.
- P22 **Dinoflagellate/cyanobacteria consortia in the tropical Indian Ocean and the north-west Australian Sea**
Tarangkoon, Woraporn¹; Hansen, Gert²; Hansen, Per¹.
1. Marine biological laboratory, University of Copenhagen, Helsingør, Denmark. 2. Department of Biology, Section of Phycology, University of Copenhagen, Copenhagen, Denmark.
- P23 **Characterization of the endosymbiotic dinoflagellates from soriticean foraminifera**
Lee, John^{1,4}; Cervasco, Megan^{1,4}; Morales, Jorge²; Billick, Morgan¹; Vessel, Eugene¹; Fimiarz, Daniel³; Hall, Tricia¹; Medior, Geraldo¹.
1. Biology, City College of New York, New York, NY, USA. 2. Interdepartmental Electron Microscopical Facility, City College of CUNY, New York, NY, USA. 3. Confocal Microscopical Facility, City College of CUNY, New York, NY, USA. 4. Invertebrates, American Museum of Natural History, New York, NY, USA.
- P24 **Ultrastructural characterization of the lytic cycle of an intra-nuclear virus of *Chaetoceros wighamii* from Chesapeake Bay, USA**
Eissler, Yoanna¹; Wang, Kui²; Coats, D. Wayne¹.
1. SERC, Edgewater, MD, USA. 2. Center of Marine Biotechnology, University of Maryland, Baltimore, MD, USA.
- P25 **High CO₂, UV, and carbon partitioning in *Thalassiosira pseudonana***
Franklin, Linda¹; Sobrino, Cristina¹; Neale, Patrick¹; Stojkovic, Slobodanka²; Beardall, John².
1. Smithsonian Environmental Research Center, Edgewater, MD, USA. 2. School of Biological Sciences, Monash University, Clayton, VIC, Australia.
- P26 **Intra- and interspecies differences in *Pseudo-nitzschia* ecophysiology, genetics and toxicity**
Thessen, Anne¹; Bowers, Holly²; Stoecker, Diane¹.
1. Horn Point Laboratory, University of Maryland Center for Environmental Science, Cambridge, MD, USA. 2. University of Maryland, Center for Marine Biotechnology, Baltimore, MD, USA.

- P27 **Temperature and irradiance impacts on the growth, pigmentation and Photosystem II quantum yields of *Haematococcus pluvialis* (Chlorophyceae)**
Evens, Terence^{1,2}; Niedz, Randall¹; Kirkpatrick, Gary².
 1. USDA-ARS, Ft. Pierce, FL, USA. 2. Mote Marine Laboratory, Sarasota, FL, USA.
- P28 **An improved method for phycobilin extraction**
Zimba, Paul; Terry, Crystal; Mischke, Charles.
 CGRU, Agricultural Research Service, Stoneville, MS, USA.
- P29 **Comparative physiology of desert biotic crust algae (Chlorophyta) and their aquatic relatives: exploring desiccation tolerance and photoprotection**
Lewis, Louise¹; Gray, Dennis^{1,3}; Catanese, Kristina¹; Cardon, Zoe^{1,2}.
 1. Ecology & Evolutionary Biology, University of Connecticut, Storrs, CT, USA. 2. Center for Integrative Geosciences, University of Connecticut, Storrs, CT, USA. 3. Plants, Soils & Biometeorology, Utah State University, Logan, UT, USA.
- P30 **Photochemical production of H₂O₂ in buffered media reduces *Prochlorococcus* growth**
Morris, J.; Zinser, Erik.
 Microbiology, University of Tennessee, Knoxville, TN, USA.
- P31 **Traditional vs. integrated aquaculture of *Gracilaria chilensis* C.J. Bird, McLachlan & E.C. Oliveira: productivity and physiological performance**
Abreu, Maria^{1,3}; Varela, Daniel²; Henriquez, Luis²; Villarroel, Adrian²; Yarish, Charles³; Sousa-Pinto, Isabel^{1,4}; Buschmann, Alejandro².
 1. Centre for Marine and Environmental Research, Porto, Portugal. 2. Centro de Investigación i-mar, Universidad de los Lagos, Puerto Montt, Chile. 3. Department of Ecology and Evolutionary Biology, University of Connecticut, Stamford, CT, USA. 4. Botany Department, University of Porto, Porto, Portugal.
- P32 **Tyrosine phosphorylation of ciliary basal bodies in *Tetrahymena thermophila***
 Rego, Megan; Edwards, Sara; Davis, Sharisse; Shehadeh, Bashar; Hufnagel, Linda.
 Cell and Molecular Biology, University of Rhode Island, Kingston, RI, USA.
- P33 **GpMyoF, a WD40 repeat-containing myosin associated with the myonemes of *Gregarina polymorpha*.**
Heintzelman, Matthew; Mateer, Marcus.
 Biology, Bucknell University, Lewisburg, PA, USA.
- P34 **Cellulose synthase (*CesA*) genes in the red alga *Porphyra yezoensis* Ueda**
Roberts, Eric¹; Roberts, Alison².
 1. Biology, Rhode Island College, Providence, RI, USA. 2. Biological Sciences, University of Rhode Island, Kingston, RI, USA.
- P35 **Molecular cloning and gene expression analysis of nitrate reductase from the harmful marine alga, *Heterosigma akashiwo***
 Coyne, Kathryn.
 College of Marine and Earth Studies, University of Delaware, Lewes, DE, USA.

- P36 **Biochemical characterization of *Entamoeba histolytica* alcohol dehydrogenase 2 (EhADH2) and a novel *E. invadens* bifunctional alcohol dehydrogenase (EiADHE)**
Perdrizet, George; Farrell, Leanne; Espinosa, Avelina.
Biology, Roger Williams University, Bristol, RI, USA.
- P37 **Molecular mechanisms of iron acquisition by diatoms**
Pritchard, LeAnn¹; Wells, Mark²; Hughes, Margaret²; Lins, Jeremy¹; Jenkins, Bethany^{1,3}.
1. Cell and Molecular Biology, University of Rhode Island, Kingston, RI, USA. 2. Institute of Marine Science, University of California Santa Cruz, Santa Cruz, CA, USA. 3. Graduate School of Oceanography, University of Rhode Island, Narragansett, RI, USA.
- P38 **Encystment of *Acanthamoeba castellanii* involves storage proteins in the form of fragmented actins and cupin domain containing proteins as detected by two-dimensional gel electrophoresis and mass spectrometry**
Ko, Ah Ryoung; Han, Byeong Gu; Ahn, Tae In.
Department of Biological Sciences, Seoul National University, Seoul, South Korea.
- P39 **Inactivation of *Giardia* strains: comparison of test methods**
 Lenaghan, Scott¹; Dykstra, Christine²; Sundermann, Christine¹.
1. Biological Sciences, Auburn University, Auburn, AL, USA. 2. Pathobiology, Veterinary Medicine, Auburn University, Auburn, AL, USA.
- P40 **The use of cDNA libraries to investigate asexual reproduction in *Porphyra umbilicalis* (L.) Kützinger**
Blouin, Nicolas¹; Grossman, Arthur²; Brawley, Susan¹.
1. School of Marine Sciences, University of Maine, Orono, ME, USA. 2. Department of Plant Biology, Carnegie Institution of Washington, Stanford, CA, USA.
- P41 **The search for horizontal gene transfer from a heterokont alga to a kleptoplastic sea slug**
Kannan, Krishna^{1,2}; Worful, Jared²; Rumpho, Mary².
1. School of Marine Sciences, University of Maine, Orono, ME, USA. 2. Biochemistry, Microbiology and Molecular Biology, University of Maine, Orono, ME, USA.
- P42 **Inactivation of either one or both of the two genes that encode fibrillin in *Synechocystis* PCC6803 impairs growth and alters cell ultrastructure for cultures grown under high irradiance**
Cunningham, F.; Tice, A.; Gantt, E..
Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, MD, USA.
- P43 **An unusual form of growth in *Porphyra*: vegetative propagation or a form of perennation?**
Pereira, Rui^{1,2}; Kim, Jang²; Sudo, Yusuke^{2,3}; Sousa-Pinto, Isabel^{1,4}; Yarish, Charles².
1. Centre for Marine and Environmental Research, Porto, Portugal. 2. Department of Ecology and Evolutionary Biology, University of Connecticut, Stamford, CT, USA. 3. Aquaculture Section, Okinawa Prefectural Fisheries and Ocean Research Center, Okinawa, Japan. 4. Department of Botany, University of Porto, Porto, Portugal.

- P44 **High resolution SIMS imaging of cations in dinoflagellate chromosomes**
 Levi-Setti, Riccardo¹; Gavrilov, Konstantin¹; Rizzo, Peter².
 1. *Biology, Texas A&M University, College Station, TX, TX, USA.* 2. *Enrico Fermi Institute and Department of Physics, Chicago, USA.*
- P45 **Cellular localization of calcium oxalate inclusions in red and green algae**
Pueschel, Curt¹; West, John².
 1. *Biological Sciences, SUNY-Binghamton, Binghamton, NY, USA.* 2. *School of Botany, University of Melbourne, Parkville, VIC, Australia.*
- P46 **Host-cell penetration in *Amoebophrya* spp. infecting *Akashiwo sanguinea*: inhibitor and ultrastructural studies**
Miller, John; Delwiche, Charles.
CBMG, University of Maryland, College Park, MD, USA.
- P47 **Polishing of municipal wastewater effluent by an algal assemblage and production of fuel from harvested biomass**
Hare, Catherine; Robertson, Tanya; Kozlowski, John; Cohen, Michael.
Biology, Sonoma State University, Rohnert Park, CA, USA.
- P48 **Molecular assessment of Hawaiian stream periphyton diversity using a universally amplifying plastid marker**
 Chan, Yvonne²; Sherwood, Alison²; Presting, Gernot¹.
 1. *Molecular Biosciences and Bioengineering, University of Hawaii, Honolulu, HI, USA.* 2. *Botany, University of Hawaii, Honolulu, HI, USA.*
- P49 **A practical method for identification and description of microbial eukaryotes using the testate amoeba *Centropyxis* as a case study**
Lahr, Daniel^{1,2}; Lopes, Sonia².
 1. *Organismic and Evolutionary Biology, University of Massachusetts - Amherst, Amherst, USA.* 2. *Department of Zoology, University of Sao Paulo, Sao Paulo, Brazil.*
- P50 **A new benthic/epiphytic *Prorocentrum* species from the western North Atlantic?**
Maranda, Lucie¹; Morton, Steve².
 1. *Graduate School of Oceanography, University of Rhode Island, Narragansett, RI, USA.* 2. *Marine Biotoxin Program, NOAA/Hollings Marine Laboratory, Charleston, SC, USA.*
- P51 **Molecular evidence for *Chondrophyucus poiteau* var. *gemmiferus* comb. nov. (Ceramiiales, Rhodophyta) from the Mexican Caribbean. Implications for the taxonomy of the *Laurencia* complex**
Diaz Larrea, Jhoana¹; Senties G., Abel¹; T. Fujii, Mutue²; F. Pedroche, Francisco¹; C. Oliveira, Mariana³.
 1. *Hidrobiología, Universidad Autónoma Metropolitana, Distrito Federal, Mexico.* 2. *Ficología, Instituto de Botânica, São Paulo, Brazil.* 3. *Botânica, Instituto de Biociências, Universidade de São Paulo, São Paulo, Brazil.*
- P52 **Marine tube-forming diatoms and their cohabitants: a floristic survey of Canadian waters using DNA barcoding**
Hamsher, Sarah; Saunders, Gary.
Dept. of Biology, University of New Brunswick, Fredericton, NB, Canada.

- P53 **Morphological and molecular studies on the Peyssonneliaceae from Vanuatu and southeastern Australia**
Dixon, Kyatt¹; Kraft, Gerald¹; Saunders, Gary².
 1. Botany, The University of Melbourne, Melbourne, VIC, Australia. 2. Biology, University of New Brunswick, Fredericton, NB, Canada.
- P54 **Characterization of a green alga isolated from a human infection**
Yu, Jingjie; Brand, Jerry.
 MCD-Biology, Univ. of Texas at Austin, Austin, TX, USA.
- P55 **Analyses of the ubiquitous species *Chara braunii* (Charales) in Japan, based on the morphology, chloroplast and nuclear DNA sequences**
Kato, Syou¹; Sakayama, Hidetoshi²; Misawa, Hideharu¹; Sano, Satomi³; Kasai, Fumie⁴; Watanabe, Makoto⁵; Tanaka, Jiro⁶; Nozaki, Hisayoshi¹.
 1. Biological Sciences, University of Tokyo, Tokyo, Japan. 2. Life Sciences, University of Tokyo, Tokyo, Japan. 3. Funabashi Shibayama High School, Chiba, Japan. 4. Environmental Biology, National Institute for Environmental Studies, Ibaraki, Japan. 5. Structural Biosciences, University of Tsukuba, Ibaraki, Japan. 6. Oceans Sciences, Tokyo University of Marine Science and Technology, Tokyo, Japan.
- P56 **Occurrence of three different genotypes of the filamentous brown alga *Geminocarpus* (Acinetosporaceae, Ectocarpales, Phaeophyceae) in Antarctica**
Amsler, Margaret¹; Peters, Akira²; Lopez-Bautista, Juan³; Amsler, Charles¹; McClintock, James¹.
 1. Department of Biology, University of Alabama at Birmingham, Birmingham, AL, USA. 2. Station Biologique, Roscoff, France. 3. Department of Biology, University of Alabama, Tuscaloosa, AL, USA.
- P57 **Current progress towards an algal heterokont tree of life**
 Andersen, Robert³; Theriot, Edward²; Jansen, Robert¹; Cattalico, Rose Ann⁴; Rocap, Gabrielle⁵; Julius, Mathew⁶; Draisma, Stefano⁷; Kawai, Hiroshi⁸; Ruck, Elizabeth¹; Ashworth, Matt¹; Zhengqiu, Cai¹; Ong, Han⁴.
 1. Section of Intergrative Biology, University of Texas at Austin, Austin, TX, USA. 2. Texas Memorial Museum, University of Texas at Austin, Austin, TX, USA. 3. Bigelow Laboratory for Ocean Sciences, West Boothbay Harbor, ME, USA. 4. Department of Biology, University of Washington, Seattle, WA, USA. 5. School of Oceanography, University of Washington, Seattle, WA, USA. 6. Department of Biological Sciences, St. Cloud State University, St. Cloud, MN, USA. 7. Leiden University Branch, Nationaal Herbarium Nederland, Leiden, Netherlands. 8. Kobe University Research Center for Inland Seas, Kobe University, Kobe, Japan.
- P58 **A multigene approach to reconstructing euglenoid systematics**
Triemer, Richard¹; Bennett, Matthew¹; Linton, Eric²; Kwiatowski, Jan⁴; Karnkowska, Anna⁴; Zakrys, Bozena⁴; Kim, Jong Im³; Shin, Woongghi³.
 1. Department of Plant Biology, Michigan State University, East Lansing, MI, USA. 2. Department of Biology, Central Michigan University, Mt. Pleasant, MI, USA. 3. Department of Bioscience, Chungnam National University, Yuseong-Gu Daejeon, South Korea. 4. Department of Plant Systematics and Geography, University of Warsaw, Warsaw, Poland.

- P59 **Phylogeny of the Euglenales inferred from chloroplast SSU and LSU rDNA sequences**
 Shin, Woongghi; Kim, Jong Im.
Biology, Chungnam National University, Daejeon, South Korea.
- P60 **Comparisons of inshore and offshore Arctic marine picoeukaryotes**
Potvin, Marianne; Lovejoy, Connie.
Biology, Laval University, Quebec, QC, Canada.
- P61 **Phylogeny and taxonomy of the Hawaiian *Laurencia* complex (Rhodomelaceae, Rhodophyta)**
Kurihara, Akira¹; Abe, Tsuyoshi²; Kogame, Kazuhiro³; Sherwood, Alison¹.
1. Botany Department, University of Hawaii, Honolulu, HI, USA. 2. The Hokkaido University Museum, Hokkaido University, Sapporo, Japan. 3. Graduate School of Science, Hokkaido University, Sapporo, Japan.
- P62 **Phylogenetic relationship of various *Ceramium* (Ceramiaceae, Rhodophyta) from the north Pacific inferred from *rbcL* DNA sequence data**
Cho, Tae¹; Fredericq, Suzanne².
1. Marine Life Science, Chosun University, Gwangju, South Korea. 2. Biology, University of Louisiana at Lafayette, Lafayette, LA, USA.
- P63 **Molecular adaptation of gametolytic, matrix metalloproteinase 1 (MMP1) gene in *Chlamydomonas* strains**
 Verghese, Bindhu¹; Buchheim, Julie²; Buchheim, Mark¹.
1. Department of Biology, University of Tulsa, Tulsa, OK, USA. 2. Department of Anatomy and Cell Biology, Center for Health Sciences, Oklahoma State University, Tulsa, OK, USA.
- P64 **Red algal rogue *Acrochaetes*: *Rhodochorton membranaceum* and *R. subimmersum* are allied to the Palmariales**
Clayden, Susan; Saunders, Gary.
Biology, University of New Brunswick, Fredericton, NB, Canada.
- P65 **Taxonomy of *Coolia* including two new species, *Coolia minuta* sp. nov. and *Coolia novella* sp. nov. (Dinophyceae)**
Faust, Maria¹; Litaker, R.³; Vandersea, Mark³; Kibler, Steven³; Holland, William³; Tester, Patricia³.
1. U.S. National Herbarium, Smithsonian Institution, Sutland, MD, USA. 2. Center for Coastal Fisheries and Habitat Research, National Ocean Service, NOAA, Beaufort, NC, USA. 3. Center for Coastal Fisheries and Habitat Research, NOS/NOAA, Beaufort, NC, USA.
- P66 **The subaerial algae: a case for morphological convergence?**
Allali, Haj Abdeslam; Lopez-Bautista, Juan.
Biological Sciences, University of Alabama, Tuscaloosa, AL, USA.

- P67 **Molecular characterization of the red algal genus *Scinaia* (Scinaiceae, Nemaliales) from the Azorean archipelago with morphological observations on *S. interrupta***
León-Cisneros, Karla^{1,2}; Gabriel, Daniela^{1,3}; Neto, Ana¹; Fredericq, Suzanne³; Riosmena-Rodriguez, Rafael².
 1. *Centro de Investigação de Recursos Naturais (CIRN), Secção de Biologia Marinha, Departamento de Biologia, Universidade dos Açores, Ponta Delgada, Portugal.* 2. *Programa de Investigación en Botánica Marina, Departamento de Biología Marina, Universidad Autónoma de Baja California Sur, La Paz, Mexico.* 3. *Department of Biology, University of Louisiana at Lafayette, Lafayette, LA, USA.*
- P68 **Clarification of the red algal genus *Peyssonnelia* in the Gulf of Mexico, with a proposal for a new red algal order based on the Peyssonneliaceae**
Fredericq, Suzanne¹; Kravesky, David¹; Norris, James².
 1. *Dept. of Biology, University of Louisiana, Lafayette, LA, USA.* 2. *Dept. of Botany, Smithsonian Institution, Washington, DC, USA.*
- P69 **New insights in the red algal order Rhodymeniales, with special emphasis on taxa from the Gulf of Mexico**
Schmidt, William; Fredericq, Suzanne.
Biology, University of Louisiana at Lafayette, Lafayette, LA, USA.
- P70 **Examining the euglenophyte mucilaginous clade with EF1 α**
Jardeleza, Sarah¹; Farmer, Mark^{2,1}.
 1. *Plant Biology, University of Georgia, Athens, GA, USA.* 2. *Cellular Biology, University of Georgia, Athens, GA, USA.*
- P71 **An exploration of the genus *Geitlerinema* (Pseudanabaenaceae) using a combined molecular and morphological approach**
Stringfellow, Emilie; Perkerson, Ralph ; Casamatta, Dale.
Biology, University of North Florida, Jacksonville, FL, USA.
- P72 **Characterization of a mycophagous amoeba-flagellate isolated from a *Phytophthora ramorum*-infected lesion of California bay laurel**
Yamamoto, Emi¹; Mazzola, Mark²; Cohen, Michael¹.
 1. *Biology, Sonoma State University, Rohnert Park, CA, USA.* 2. *USDA Agricultural Research Service, Tree Fruits Research Laboratory, Wenatchee, WA, USA.*
- P73 **Grazing, growth, and behavioral reactions of a ciliate fed *Alexandrium* spp: apparent lack of response to saxitoxin**
Schoener, Donald; McManus, George; Avery, David; Dam, Hans.
Marine Sciences, University of Connecticut, Groton, CT, USA.
- P74 **Assembly of ribosomal DNA in *Pneumocystis***
Keely, Scott¹; Slaven, Bradley^{3,4}; Fan, David³; Smulian, A.^{2,4}; Cushion, Melanie^{2,4}; James, Stringer¹.
 1. *Molecular Genetics, University of Cincinnati, Cincinnati, OH, USA.* 2. *Cincinnati Veterans Administration Medical Center, Cincinnati, OH, USA.* , OH, USA. 3. *Biomedical Engineering, University of Cincinnati, Cincinnati, OH, USA.* 4. *Infectious Diseases, University of Cincinnati, Cincinnati, OH, USA.*

- P75 **Updated draft assembly and annotation of the *Pneumocystis carinii* genome**
Slaven, Bradley^{3,1}; Keely, Scott⁵; Fan, David^{2,7}; Meller, Jaroslaw^{2,4}; James, Stringer⁵; Smulian, Alan^{1,6}; Cushion, Melanie^{1,6}.
1. Infectious Diseases, University of Cincinnati, Cincinnati, OH, USA. 2. Environmental Health, University of Cincinnati, Cincinnati, OH, USA. 3. Biomedical Engineering, University of Cincinnati, Cincinnati, OH, USA. 4. Biomedical Informatics, Children's Hospital Research Foundation, Cincinnati, OH, USA. 5. Molecular Genetics, University of Cincinnati, Cincinnati, OH, USA. 6. Infectious Diseases, Cincinnati Veteran Affairs Medical Center, Cincinnati, OH, USA. 7. Biomedical Engineering, Duke University, Durham, NC, USA.
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THURSDAY, 9 AUGUST

6:50 – 7:50 Continental Breakfast in Rotunda

Salon III-V, Thursday Morning:

PSA Special Session: Energetic and Elemental Stoichiometries in Phytoplankton Ecology: Ecology and Evolution

Introducing and Presiding: Paul Kugrens, Colorado State University

8:20 PLENARY LECTURE: Why do phytoplankton make such a fuss about carbon dioxide?
Raven, John.

College of Life Sciences, University of Dundee, Dundee, United Kingdom.

9:20 Size matters: macroevolutionary patterns in marine diatoms and dinoflagellates
Finkel, Zoe.

Environmental Science, Mount Allison University, Sackville, NB, Canada.

9:50 BREAK

PSA Contributed Papers: Phylogenetics and Taxonomy 4

Presiding: Morgan Vis, Ohio University

10:20 The genus *Chondria* (Rhodomelaceae, Ceramiales) in the Gulf of Mexico
Ehrenhaus, Constanza; Fredericq, Suzanne.

Biology, university of Louisiana at Lafayette, Lafayette, LA, USA.

10:40 A morphological and molecular investigation of the genus *Botryocladia* (Rhodophyta, Rhodymeniaceae) in Bermuda, western Atlantic
Schneider, Craig¹; Lane, Christopher².

1. Department of Biology, Trinity College, Hartford, CT, USA. 2. Department of Biochemistry and Molecular Biology, Dalhousie University, Halifax, NS, Canada.

- 11:00 **Untangling cyanobacterial systematics: erection of *Emicolynghya* gen. nov. from a polyphyletic clade of *Leptolynghya***
Perkerson, Ralph¹; Johansen, Jeffrey²; Kovacik, Lubomir³; Casamatta, Dale¹.
 1. *University of North Florida, Jacksonville, FL, USA.* 2. *Department of Biology, John Carroll University, University Heights, OH, USA.* 3. *Dept. Botany, Comenius University, Revova, Slovakia.*
- 11:20 **Origin of the cyanobacterial *gnd* gene in secondary phototrophs and non-photosynthetic protists**
Maruyama, Shinichiro¹; Iseki, Mineo²; Watanabe, Masakatsu²; Nozaki, Hisayoshi¹.
 1. *Department of Biological Sciences, University of Tokyo, Tokyo, Japan.* 2. *Department of Photoscience, Graduate University for Advanced Studies, Kanagawa, Japan.*
- 11:40 **LUNCH BREAK**
- 12:00 – 1:00 **Ticketed (pre-paid) lunches, Rotunda**

Salon I-II, Thursday Morning:

PSA Contributed Papers: Cellular and Molecular Biology 2

Presiding: Senjie Lin, University of Connecticut, Groton

- 10:20 **Algae-derived isoprenoid biosynthesis pathway suggesting a plastid in an oyster parasite, *Perkinsus marinus***
Matsuzaki, Motomichi¹; Kuroiwa, Tsuneyoshi²; Kita, Kiyoshi³; Nozaki, Hisayoshi¹.
 1. *Department of Biological Sciences, Graduate School of Science, The University of Tokyo, Tokyo, Japan.* 2. *Department of Life Science, College of Science, Rikkyo University, Tokyo, Japan.* 3. *Department of Biomedical Chemistry, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan.*
- 10:40 **Preliminary study of differential gene expression during growth and toxin production in *Prymnesium parvum* (Haptophyta)**
 Talarski, Aimee; La Claire, John.
MCD Biology, U. Texas at Austin, Austin, TX, USA.
- 11:00 **The consequences of genome reduction in eukaryotes inferred from nucleomorph comparative genomics**
Lane, Christopher¹; van den Heuvel, Krystal¹; Curtis, Bruce²; Fong, Anna¹; Kozera, Catherine²; Parsons, Byron²; Bowman, Sharen²; Archibald, John¹.
 1. *Biochemistry and Molecular Biology, Dalhousie University, Halifax, NS, Canada.* 2. *The Atlantic Genome Centre, Halifax, NS, Canada.*
- 11:20 **Complete sequence and analysis of the mitochondrial genome of *Hemiselmis andersenii* CCMP644 (Cryptophyceae)**
Kim, Eunsoo; Lane, Christopher; Archibald, John.
Biochemistry, Dalhousie University, Halifax, NS, Canada.
- 11:40 **LUNCH BREAK**

12:00 – 1:00 Ticketed (pre-paid) lunches, Rotunda

Bristol A-B, Thursday Morning:

ISOP Contributed Papers: Diversity\Phylogeny

Presiding: John C. Clamp, North Carolina Central University

8:00 **Opening Remarks**

8:10 **A 2 year longitudinal study of *Cryptosporidium* species and genotypes in dairy cattle**

Fayer, Ronald; Santin, Monica; Trout, James.

USDA, Beltsville, MD, USA.

8:30 **Genetic diversity of a malaria parasite, *Plasmodium mexicanum*: changes in diversity over time and space and effects on parasite life history traits**

Vardo, Anne; Schall, Joseph.

Biology, University of Vermont, Burlington, VT, USA.

8:50 **Labyrinthulomycetes diversity in temperate estuaries**

Collado, Enixy; Collier, Jackie.

Marine Science Reserch Center, Stony Brook University, Stony Brook, NY, USA.

9:10 **Placement of diverse amoeboid lineages in the eukaryotic tree of life and the evolution of ‘amoebozoa’**

Tekle, Yonas; Grant, Jessica; Katz, Laura .

Biological Science, Smith Collge, Northampton, USA.

9:30 **Phylogeny and comparative morphology of marine interstitial cercozoans**

Chantangsi, Chitchai¹; Leander, Brian².

1. Zoology, University of British Columbia, Vancouver, BC, Canada. 2. Botany and Zoology , University of British Columbia, Vancouver, BC, Canada.

9:50 **BREAK**

10:20 **Morphological evolution of the *Corallomyxa*, foraminifera, *Gromia*, haplosporidia clade**

Wegener Parfrey, Laura¹; Tekle, Yonas²; Grant, Jessica²; Bowser, Samuel^{3,4}; Katz, Laura^{2,1}.

1. Program in Organismic and Evolutionary Biology, Universtiy of Massachusetts - Amherst, Amherst, USA. 2. Department of Biology, Smith College, Northampton, USA. 3. Department of Biomedical Sciences, State University of New York at Albany, Albany, NY, USA. 4. Wadsworth Center, New York State Department of Health, Albany, NY, USA.

10:40 **Microbial observatory in the Cariaco basin – dynamics of protistan diversity across time, space, and chemical gradients**

Edgcomb, Virginia¹; Jeon, Sunok²; Taylor, Gordon³; Orsi, William²; Leslin, Chesley²; Bunge, John⁴; Epstein, Slava².

1. Geology and Geophysics, Woods Hole Oceanographic Institution, Woods Hole, MA, USA. 2. Marine Sciences Center, Northeastern University, Nahant, USA. 3. Marine Sciences

Research Center, Stony Brook University, Stony Brook, NY, USA. 4. Social Statistics, Cornell University, Ithaca, NY, USA.

- 11:00 **Diversity of oligotrich and choreotrich ciliates in nearshore sediments and plankton**
Tamura, Maiko¹; Doherty, Mary²; Jaris, Hannah¹; Costas, Barbara³; McManus, George³; Katz, Laura^{1,2}.
1. Biological Sciences, Smith College, Northampton, MA, USA. 2. Organismic and Evolutionary Biology, UMass-Amherst, Amherst, MA, USA. 3. Marine Sciences, University of Connecticut, Groton, CT, USA.
- 11:20 **Molecular phylogeography of peritrichous ciliates**
Gentekaki, Eleni; Lynn, Denis.
Integrative Biology, University of Guelph, Guelph, ON, Canada.
- 11:40 **New perspectives on the phylogenetic relationships of sessilid peritrichs: are morphologically ‘distinct’ taxa really phylogenetically distinct?**
Clamp, John; Williams, Daniel.
Biology, N.C. Central Univ., Durham, NC, USA.

12:00 **LUNCH BREAK**

12:00 – 1:00 **Ticketed (pre-paid) lunches, Rotunda**

Salon III-V, Thursday Afternoon:

PSA Contributed Papers: Phylogenetics and Taxonomy 5

Presiding: Richard Triemer, Michigan State University

- 1:30 **Phylogeny of the euglenoid loricate genera *Trachelomonas* and *Strombomonas* (Euglenophyta) inferred from nuclear SSU and LSU rDNA**
Ciugulea, Ionel¹; Nudelman, Maria¹; Brosnan, Stacy²; Triemer, Richard¹.
1. Michigan State University, East Lansing, MI, USA. 2. Division of Life Sciences, Rutgers University, Piscataway, NJ, USA.
- 1:50 **Phylogeography, morphological variation and taxonomy of the toxic dinoflagellate *Gambierdiscus toxicus* (Dinophyceae)**
Richlen, Mindy¹; Morton, Steve²; Barber, Paul³.
1. Biology, Boston University, Boston, USA. 2. Marine Biotoxin Program, NOAA/NOS, Charleston, SC, USA. 3. Biology, Boston University, Boston, USA.
- 2:10 **Assesing DNA barcodes as an identification tool in dinoflagellates**
Stern, Rowena; Keeling, Patrick.
Botany, University of British Columbia, Vancouver, BC, Canada.
- 2:30 **Character evolution in dinoflagellates with complex organelles**
Hoppenrath, Mona; Leander, Brian.
Dept of Botany, University of British Columbia, Vancouver, BC, Canada.

2:50 **A taxonomic study of a *Protoberidinium oblongum*-complex and establishment of cultures of *Protoberidinium* with non-cellular food items**
Yamaguchi, Aika¹; Kawamura, Hiroshi²; Horiguchi, Takeo³.
1. Center for Advanced Marine Core Research, Kochi University, Nankoku, Japan. 2. Management International, Integrated Ocean Drilling Program, Sapporo, Japan. 3. Natural History Sciences, Hokkaido University, Sapporo, Japan.

3:10 **BREAK**

3:30 – 5:00 ***Phyco-Speed Dating***

Presiding: Richard McCourt, National Science Foundation

Presenters TBA

Salon I-II, Thursday Afternoon:

PSA Contributed Papers: Ecology and Population Biology 4

Presiding: Dennis Hanisak, Harbor Branch Oceanographic Institution

1:30 **Variable abundance of *Karlodinium veneficum* in US east coast as detected by a dual-gene real-time PCR assay**

Zhang, Huan; Lin, Senjie.

Marine Sciences, University of Connecticut, Groton, CT, USA.

1:50 **Natural vs. anthropogenic nitrogen uptake in *Ulva* and *Gracilaria*, two bloom-forming macroalgae**

Thornber, Carol¹; DiMilla, Peter²; Nixon, Scott²; McKinney, Richard³.

1. Biological Sciences, University of Rhode Island, Kingston, RI, USA. 2. Graduate School of Oceanography, University of Rhode Island, Narragansett, RI, USA. 3. Atlantic Division, U.S. E.P.A., Narragansett, RI, USA.

2:10 **Palatability of *Palmaria decipiens* and its endo/epiphyte *Elachista antarctica* to three common antarctic amphipods**

Bucolo, Anthony¹; Amsler, Charles¹; McClintock, James¹; Baker, Bill².

1. Biology, University Alabama Birmingham, Birmingham, AL, USA. 2. Chemistry, University of South Florida, Tampa Bay, FL, USA.

2:30 **Discovery of secondary cell walls and lignin precursors in the joints of the articulated coralline alga *Calliarthron***

Martone, Patrick¹; Estevez, Jose²; Ralph, John³; Lu, Fachuang³; Ruel, Katia⁴; Denny, Mark¹; Somerville, Chris².

1. Hopkins Marine Station, Stanford University, Pacific Grove, CA, USA. 2. Carnegie Institute of Washington, Stanford University, Stanford, CA, USA. 3. University of Wisconsin, Madison, WI, USA. 4. Centre National de la Recherche Scientifique, Paris, France.

2:50 **Seaweed biodiversity of the Gulf of California estimated from a century of historical records**
Zertuche-Gonzalez, Jose¹; Pacheco-Ruiz, Isai¹; Galindo-Bect, Luis¹; Galvez-Telles, Alberto¹; Riosmena-Rodriguez, Rafael².
1. *Marine Botany, Instituto de Investigaciones Oceanológicas UABC, Ensenada, Mexico.* 2. *Marine Biology, UABCS, La Paz, Mexico.*

3:10 **BREAK**

3:30 – 5:00 ***Phyco-Speed Dating*** – In Salon III-V

Bristol A-B, Thursday Afternoon:

ISOP Past President Address

Introducing and Presiding: Lea Bleyman, Baruch College

1:30 **Lost among the alveolates**

Coats, D. Wayne

Smithsonian Environmental Research Center, Edgewater, MD, USA

ISOP Platform Session: Ecology

Presiding: Gaytha A. Langlois, Bryant University

2:30 **Algal contributions to the ancient organic carbon cycle**

Kodner, Robin; Knoll, Anderw.

Organismic and Evolutionary Biology, Harvard University, Cambridge, MA, USA.

2:50 **Development of a real-time quantitative PCR assay for the thraustochytrid QPX (Labyrinthulomycota)**

Liu, Qianqian; Allam, Bassem; Collier, Jackie.

Marine Sciences Research Center, Stony Brook University, Stony Brook, NY, USA.

3:10 **BREAK**

3:40 **Reevaluation of the role of naked, amoeboid protists in bacterivory and microbial carbon flux in coastal marine and estuarine waters**

Juhl, Andrew¹; Anderson, O. Roger¹; Lesen, Amy².

1. *Lamont-Doherty Earth Observatory, Columbia University, Palisades, NY, USA.* 2. *Pratt Institute, Brooklyn, NY, USA.*

- 4:00 **Effects of temperature, iron and CO₂ on microzooplankton assemblages in the Ross Sea, Antarctica and the North Atlantic spring bloom**
Rose, Julie¹; Feng, Yuanyuan²; Gobler, Chris³; Gutierrez, Robert³; Hare, Clinton⁴; Leblanc, Karine⁶; DiTullio, Jack⁵; Hutchins, David².
1. Biology, Woods Hole Oceanographic Institution, Woods Hole, USA. 2. Marine Environmental Biology, University of Southern California, Los Angeles, CA, USA. 3. Marine Sciences Research Center, Stony Brook University, Stony Brook, NY, USA. 4. College of Marine Studies, University of Delaware, Lewes, DE, USA. 5. Hollings Marine Laboratory, College of Charleston, Charleston, SC, USA. 6. University of the Mediterranean, Marseille, France.
- 4:20 **Density, carbon content, and potential atmospheric yield of respiratory carbon dioxide of non-testate amoebae in a terrestrial bryophyte community: ecological and biogeochemical implications**
Anderson, O. Roger.
Biology, Lamont-Doherty Observatory, Columbia University, Palisades, NY, USA.
- 4:40 **Using protists to teach evolution**
Farmer, Mark.
Cellular Biology, University of Georgia, Athens, GA, USA.

Grand Ballroom, Thursday Evening:

6:00 to 6:30 Cocktails (in Grand Foyer)

6:30 to 9:30 ***PSA-ISOP Banquet and Awards Ceremony***

Friday, 10 AUGUST

Optional Intertidal Field Trip

Guides: Brian Wysor, Roger Williams University and Carol Thornber, University of Rhode Island

The field trip leaves from front of the hotel at 9:00 AM and will return at 3:00 PM.

Abstracts

ABSTRACTS NUMBERS ARE VERY CLOSE TO ABSTRACT PRESENTATION ORDER .
SEE AUTHOR INDEX ON PAGE 124 TO FIND SPECIFIC ABSTRACTS.

1

THE DISTRIBUTION OF PLASTIDS AMONG EUKARYOTES: PAST, PRESENT, AND FUTURE

Delwiche, Charles

Cell Biology and Mol. Genetics, University of Maryland, College Park, MD, USA

Chloroplasts are the unifying thread that links together all algae. Their endosymbiotic origin from previously free-living cyanobacteria is well demonstrated, as is the spread of plastids among eukaryotes via secondary and tertiary endosymbiosis. Much more controversial are the relationships among those lineages that contain plastids, and the inferred history of plastid gain and loss that the relationships would imply. One of the key characteristics of endosymbiotic organelles is the ability to import polypeptides from the cytosol, with the concomitant transfer of endosymbiont genes to the host genome. The advent of genomic data has made it possible to trace individual gene phylogenies within the context of such complex, chimeric genomes, and has led to the identification of a number of putative plastid genes in non-photosynthetic organisms. However, to interpret such observations requires assumptions concerning the relative timing of symbiosis and gene transfer, and contrasting models can be identified with distinctly different predictions for such timing. Understanding predation, parasitism, and non-plastid sym-bioses may help refine such models and clarify the likely history of plastid evolution.

2

ORGANELLE RETENTION IN THE PHOTOSYNTHETIC CILIATE *MYRIONECTA RUBRA*

Johnson, Matthew

Institute of Marine and Coastal Sciences, Rutgers University, New Brunswick, NJ, USA

The photosynthetic ciliate *Myrionecta rubra* is ubiquitous in coastal and estuarine waters, and capable of forming massive and highly productive red tides. *M. rubra* is well documented to feed on cryptophyte algae, and while some strains sequester and enslave organelles from their prey, others may have a permanent endosymbiont and feed only to acquire growth factors. We have studied an Antarctic strain of *M. rubra* as a model for describing organelle sequestration, carbon metabolism, and organelle evolution. We found that this *M. rubra* strain steals chloroplasts from the cryptophyte *Geminigera cryophila*, sequestering them with prey mitochondria and cytoplasm within numerous membrane-bound complexes. These complexes can be replicated, but only when the prey nucleus is present or recently lost. The retained prey nucleus is transcriptionally active for weeks after sequestration, but is incapable of division within the ciliate. Maximum growth and photosynthetic rates, pigment concentrations, and chloroplasts per cell occurred when a prey nucleus was present, while all of these parameters declined substantially with starvation. *M. rubra* functions as a phototroph by constant reestablishment of its chimeric cytostructure, and thus is an interesting evolutionary model for organelle acquisition independent of symbiosis.

3

SYMBIODINIUM-CNIDARIAN SYMBIOSES: ONTOGENY AND DIVERSITY

Coffroth, Mary Alice¹, Voolstra, Christian² & Medina, Mónica²

¹*Geological Sciences, University at Buffalo, Buffalo, NY, USA;* ²*University of California at Merced, Merced, CA, USA*

The endosymbiosis of the dinoflagellate *Symbiodinium* spp. with cnidarians is one of the most striking and ecologically important relationships in the marine environment, having profound effects on the host's physiology and ecology. While some coral species can simultaneously harbor multiple algal strains, these are only a subset of all strains that are known and most corals harbor a single algal type. Additionally, these strains are not distributed randomly among host species, but show a high level of host-specificity. Since a majority of corals acquire symbionts horizontally, the question arises as to how and when is this specificity established. We followed the early ontogeny of the cnidarian-*Symbiodinium* symbiosis in scleractinian and octocorals.

Primary polyps placed over a range of habitats acquire multiple *Symbiodinium* clades in addition to that normally harbored by the host. Further experiments demonstrated that newly settled polyps continued to acquire symbionts for at least several weeks after metamorphosis and the symbionts type initially acquired did not affect early survival of the newly settled polyp. However, after several months symbiont diversity was reduced and the juvenile coral harbored only the symbiont type found in the adult. Preliminary gene expression studies confirm the relative non-selectivity of initial acquisitions.

4

CONVERGENT EVOLUTION IN EUKARYOTIC CELLS

Leander, Brian S.

Zoology & Botany, University of British Columbia, Vancouver, BC, Canada

Convergent evolution refers to the independent development of similar traits in distantly related organisms, usually in response to similar selective pressures in similar environments. I will address the pervasiveness of reoccurring morphologies in microeukaryotes by presenting some working definitions of relevant terms and a succession of compelling examples of convergent evolution at the cellular level. An introduction to the concept of “residual capacity,” (i.e. the influence of ancestral constraints on subsequent evolution) will enable me to distinguish between three broad and overlapping categories of convergence: “parallel”, “proximate” and “ultimate”. I will propose that ultimate convergence is common in microbial eukaryotes because of the relatively large phylogenetic distances that exist between different microbial lineages having limited repertoires of morphological characters. The examples of convergent evolution that I present will be separated into four general categories: (1) multicellular analogues, (2) subcellular analogues, (3) subcellular analogues to multicellular systems and (4) multicellular analogues to subcellular systems. I will focus largely on subcellular analogues and present examples from our own research on the biodiversity of microeukaryotes in marine interstitial environments, planktonic environments and the intestinal environments of metazoan hosts. These examples will also highlight convergent evolution in the structural organization of symbiotic relationships between ectobiotic bacteria and protozoan hosts that thrive in semi-anaerobic environments. Moreover, I will discuss similar traits found in both protozoan parasites and metazoan parasites that appear to represent examples of subcellular analogues to multicellular systems. The talk will conclude by listing several challenging topics relating to the study of convergent evolution, such as (1) distinguishing between convergence from morphostasis, (2) understanding the selective forces operating at microbial scales and (3) understanding the developmental patterns and processes associated with complex ultrastructural systems in eukaryotic cells.

5

PRIMARY AND SECONDARY BACTERIAL SYMBIONTS OF *EUPLOTES*: A COMPLEX NET OF EVOLUTIONARY AND ADAPTATIVE PHENOMENA

Petroni, Giulio

Department of Biology, University of Pisa, Pisa, Italy

It is known since decades that members of the genus *Euplotes* (Ciliophora) can harbor endosymbiotic bacteria. Many of these symbiotic associations are occasional but, in some cases, permanent associations have been reported. The best studied example is represented by *Polynucleobacter necessarius* (Betaproteobacteria, Burkholderiaceae) that was first described as endosymbiont of *Euplotes aediculatus*. Further studies showed that the *Polynucleobacter-Euplotes* association is an obligatory symbiosis between a monophyletic group of *Euplotes* species and bacteria belonging to the genus *Polynucleobacter*. In this system, neither the host nor the symbiont is able to survive independently. Recent studies revealed the existence of free-living populations of *Polynucleobacter necessarius*-like bacteria that are phylogenetically closely related to the endosymbiotic ones but, apparently, are not able to establish symbiotic associations with *Euplotes*. On the other side, phylogenetic analyses based on 16S/18S rRNA data suggest that *Euplotes*, during its evolution, recruited *Polynucleobacter* as symbiont more than once. Moreover, in two cases, we could observe the functional replacement of *Polynucleobacter* symbionts by other bacteria that also belong to the family Burkholderiaceae. These bacteria likely represent a new genus for which no free-living members have been so far described. At present, the genomes of two *Polynucleobacter* strains, one obligatory free-living and one obligatory endosymbiotic, are going to be completely sequenced. The comparative analysis of these two genomes will provide unique insights in the evolutionary adaptations taking place during the early phase of endosymbiosis. The obtained

data will also contribute to shed light on the metabolic interactions between *Euplotes* and its *Polynucleobacter* symbionts. Beside *Polynucleobacter*, this group of *Euplotes* can harbor additional symbionts. These secondary bacterial endosymbionts represent either species previously reported from other ciliates or completely new species. In most cases the functional role of these associations still waits to be clarified.

6

DIATOM BLOOMS, CHYTRID EPIDEMICS AND THE EVOLUTIONARY ECOLOGY OF ITS INTERACTIONS

Ibelings, Bastiaan^{1,2}, De Bruin, Arnout², Kagami, Maiko^{3,2} & Van Donk, Ellen²

¹*Aquatic Ecology, Eawag, Kastanienbaum, Switzerland;* ²*Foodweb Studies, NIOO, Nieuwersluis, Netherlands;*

³*Environmental Science, Toho University, Funabashi, Japan*

Coevolution is a widespread process that has shaped many of the interactions we study in ecology. Yet coevolution in action is hard to detect. Based upon their intimate relationship and large reciprocal fitness costs, host and parasites show great promise for the study of coevolution. We study interactions between the diatom *Asterionella formosa* as a host and the chytrid *Zygorhizidium planktonicum* as a parasite. Prevalence of infection reaches epidemic proportions in bi-yearly epidemics that terminate the diatom blooms. Refuges for *Asterionella* on the sediment of the lakes (where the parasite is not infective) appear important in stabilizing the host-parasite relationship. Experiments showed that the outcome of the infection process was dependent on both the genotype of the host and parasite. No overall infective hosts or overall resistant parasites were detected. Both findings emphasize the potential for coevolution between *Asterionella* and *Zygorhizidium*. Hosts may limit negative fitness effects by maintaining genetic diversity. This prohibits adaptation of the parasite to locally common genotypes. Indeed genetic diversity of *Asterionella* in lakes where chytrids are abundant exceeded diversity in a lake with sporadic infection. The protection offered by genetic diversity was demonstrated in experimental evolution. *Zygorhizidium* rapidly improved its fitness on monoclonal *Asterionella*, but failed to do so in multiclonal populations of its host diatom. A final field of interest to us is the role of parasites in lake foodwebs. The abundant zoospores are excellent food for zooplankton and epidemics overcome food shortage during blooms of large inedible diatoms.

7

THE EFFECTS OF *IN SITU* AND MESOCOSM NUTRIENT ADDITIONS ON THE EPIPHYTIC ALGAL COMMUNITY OF *VALLISNERIA AMERICANA* FROM THE LOWER ST. JOHNS RIVER, FLORIDA

Dunn, Angela E.¹, Dobberfuhl, Dean² & Casamatta, Dale¹

¹*Department of Biology, University of North Florida, Jacksonville, FL, USA;* ²*St Johns River Water Management District, Palatka, FL, USA*

Epiphytes are algae found attached and living on submersed vegetation, and can greatly alter the growth of submerged aquatic vegetation (SAV), which in turn influences many aspects of the river ecosystem. To understand the role of epiphytes in the St. Johns River, *in situ* permanent experimental plots containing the common SAV *Vallisneria americana* were demarcated and amended with nitrogen and phosphorus. Concurrently, mesocosms containing *V. americana* were set up with three levels of nitrogen and phosphorus. Both experimental units were monitored on a monthly basis for one year by collecting and analyzing the epiphyte communities, chlorophyll *a* values and SAV growth. Differences in the algal communities of the control vs. experimental plots were noted, as well as differences in the overall algal abundance. Diversity and biomass of the chlorophytes of the *in situ* experimental setup increased in the fertilizer treatment, though there was not a significant ($p > 0.05$) difference between treatments. Similarly, diatom and cyanobacterial communities remained similar throughout the monitoring. Epiphyte community diversity in the mesocosms was not significantly ($p > 0.05$) different, but nitrogen amendments promoted significant ($p < 0.05$) cyanobacterial growth, while increases in phosphorus promoted significant ($p < 0.05$) chlorophyte growth. Thus, nutrient additions to the St. Johns River may have adverse effects on the SAV community as a whole.

8

YOU CAN'T JUDGE A BOOK BY LOOKING AT THE COVER: UNDERSTANDING BIODIVERSITY OF THE CONJUGATING GREEN ALGAE

Hall, John D.

Cell Biology and Molecular Genetics, University of Maryland, College Park, MD, USA

The conjugating green algae are a large group of structurally diverse freshwater algae. These organisms are important primary producers in many freshwater habitats and can survive in some seemingly extreme habitats. Their close relationship to land plants makes them excellent models for plant processes, and evolution of their structural and developmental characteristics provides insight into the evolution of early land plants. Phylogenetic and structural investigations of the conjugating green algae suggest that they are perhaps even more structurally diverse than is indicated by their sometimes delicately ornamented cell walls. Molecular phylogenetic analyses suggest that there have been a number of transitions in growth form (from unicellular to filamentous or the reverse), and that there are some fundamental differences in such critical processes as cell division among these organisms. These characteristics and their importance for understanding zygnematophyte diversity and evolution are discussed.

9

ARE *ANTITHAMNION PECTINATUM*, *CENTROCERAS CLAVULATUM* AND *SPYRIDIA FILAMENTOSA* (RHODOPHYTA) COSMOPOLITAN RED ALGAL SPECIES?

Won, Boo Y.¹, Cho, Tae O.² & Suzanne, Fredericq¹

¹*Biology, University of Louisiana at Lafayette, Lafayette, LA, USA;* ²*Marine Life Science, Chosun University, Gwangju, South Korea*

Three red algal species in the Ceramiaceae (Cerariales) have been described in the phycological literature as being widespread in distribution. Instead, studies based on comparative morphology, an examination of historical specimens, and molecular evidence inferred from chloroplast-encoded *rbcL*, or nuclear LSU rDNA and SSU rDNA sequence analyses, point to the opposite. *Antithamnion pectinatum* is restricted to New Zealand, and is distinct from *A. hubbsii* from California and from *A. nipponicum* from Japan, which are conspecific taxa. The distribution of *A. nipponicum* is extended to California, Atlantic North Carolina, and the Mediterranean Sea; historical reports suggest that *A. nipponicum* was recently introduced from Japan. *Centroceras clavulatum*, described from Peru, is restricted to that country, N. Chile and S. California; there at least four additional species going under the name *C. clavulatum* that are either new to science or that have been placed under its synonymy. *Spyridia filamentosa*, described from the Adriatic Sea, is restricted to that region, E. Florida and the Caribbean Sea; three well-supported clades each encompass distinct species that are likewise either new or validly described taxa that were placed in synonymy under that name. It has become apparent that many species referred to in the literature as "cosmopolitan" have in fact a more restricted distribution, and that the term "cryptic" species is incorrect as applied to these taxa. Truly cosmopolitan macroalgal species may only be those that are non-native and invasive in a particular region.

10

MOLECULAR ANALYSIS ON THE MATING TYPE LOCUS OF *GONIUM PECTORALE* (VOLVOCALES, CHLOROPHYTA)

Hamaji, Takashi¹, Takahashi, Fumio², Nishii, Ichiro³ & Nozaki, Hisayoshi¹

¹*Department of Biological Science, Graduate School of Science, University of Tokyo, Bunkyo-ku, Japan;*

²*Department of Biomolecular Sciences, Graduate School of Life Sciences, Tohoku University, Sendai-shi, Japan;* ³*Frontier Research System, RIKEN, Wako-shi, Japan*

The volvocine or colonial volvocalean algae are a model lineage to study the evolution of sexual reproduction. *Gonium pectorale* is an isogamous volvocine alga that has flattened 8- or 16-celled colonies. Only one of the two conjugating isogametes of *Chlamydomonas reinhardtii* has a tubular mating structure (TMS), while *G. pectorale* has bilateral mating papilla -- each of the two isogametes bears a TMS. *C. reinhardtii* has a mating type (MT) locus harboring several mating type-specific genes involved in mating type determination and/or formation of TMS. Studies of the *G. pectorale* MT locus could be very informative in terms of "sex evolution," because the evolution of anisogamy/oogamy might have occurred in an ancestral isogamous organism with bilateral mating papillae (Nozaki et al. 2000, MPE). In this study, as the first step to identify the

G. pectorale MT locus, we isolated from *G. pectorale* an orthologue of *C. reinhardtii* MT determining minus-dominance (*CrMID*) gene (Ferris and Goodenough 1997, Genetics), which is localized only in *C. reinhardtii* minus MT locus. 3' and 5' RACE RT-PCR using degenerate primers based on *MID* genes of *C. reinhardtii*, *C. incerta* (Ferris et al. 1997, PNAS), and *Pleodorina starrii* (Nozaki et al. 2006, Curr. Biol.) discovered a *CrMID*-orthologous 164 AA-coding gene (*GpMID*). *GpMID* contained a leucine-zipper RWP-RK domain near the C-terminal, as is the case with *CrMID*. Three out of four *GpMID* introns were located at the same places as three *CrMID* introns. Southern blotting showed that *GpMID* was coded only in one of two MTs of *G. pectorale*. RT-PCR revealed that *GpMID* expression increased during nitrogen-starvation. Analysis of F1 progenies showed that mating phenotype and presence/absence of *GpMID* correlated with each other. In addition, another MT-specific gene *GpMTD1*, a homologue of *C. reinhardtii* *MTD1* (Ferris et al. 2002, Genetics), was identified in MT- strain of *G. pectorale*. Inheritance and expression of this gene is essentially the same as those of *GpMID*. Difference between *C. reinhardtii* and *G. pectorale* in orientation of *MID* and *MTD1* implies drastic genomic conversion occurring in the MT locus.

11

A PHYLOGENETIC AND MORPHOMETRIC STUDY OF THE FRESHWATER GREEN ALGA *PEDIASTRUM DUPLEX* (SPHAEROPLEALES, CHLOROPHYCEAE)

McManus, Hilary A.

Ecology and Evolutionary Biology, University of Connecticut, Storrs, CT, USA

Accurate recognition of green algal species is essential for ecological studies, estimates of biodiversity, reconstructing past climates, and biotechnology. This study combined molecular and morphological data to resolve evolutionary relationships among the genera of the freshwater family Hydrodictyaceae (Chlorophyta), and tested the boundaries of *Pediastrum duplex* Meyen 1829. Molecular phylogenetic analyses of nuclear large subunit (26S rDNA) and chloroplast RuBisCo (*rbcL*) sequence data were performed on 103 ingroup isolates, 44 of which were from culture collections representing North America and Europe, and 59 were wild isolates from North America, Europe and Australia. The individual data sets, representing two cellular compartments, produced similar topologies so therefore were combined for maximum likelihood and Bayesian analyses. The resulting two-gene phylogenies revealed the *P. duplex* morphotype is polyphyletic, recovered in three distinct lineages. A landmark-based morphometric analysis was performed to determine if the three lineages possessing the *P. duplex* morphotype were distinguishable. One of the groups was shown to be morphologically distinct, supporting its separation from the other two *P. duplex* groups in the molecular tree. Erection of a new genus is recommended for this group. Multiple lineages of the *P. duplex* morphotype may be the result of a retained plesiomorphic morphology that is expressed in distinct parts of the phylogeny, multiple lineages converging on a similar morphology, or the inability of phycologists to detect differences using common methodology. This study supports the conclusions of other recent investigations of microscopic eukaryotes, that the morphospecies concept is inadequate for accurately recognizing species lineages in these taxa. The diversity of these organisms is poorly captured and vastly underestimated by phenotype alone, therefore the inclusion of additional forms of data is integral for accurate assessments of species boundaries and biodiversity.

12

UTILIZING AN INTEGRATIVE TAXONOMIC APPROACH OF MOLECULAR AND MORPHOLOGICAL CHARACTERS TO DELIMIT SPECIES IN THE RED ALGAL FAMILY KALLYMENIACEAE (RHODOPHYTA)

Clarkston, Bridgette E. & Saunders, Gary

University of New Brunswick, Fredericton, NB, Canada

Along the coast of British Columbia, Canada, the majority of species in the red algal family Kallymeniaceae (Rhodophyta) are taxonomically challenging. The morphological and anatomical traits traditionally used for identification are often subject to variability, making species discrimination difficult. As such, there are potentially many cryptic species in the flora that have been overlooked. We utilize a taxonomic approach that involves both molecular and morphological characters to delimit species. While various molecular markers have been shown to resolve algal taxonomy effectively, we use the DNA barcode (*coxI-5'*) because: 1) it is sufficiently variable to discriminate between even closely related species of red algae; 2) it is a rapid and inexpensive molecular tool; and 3) our data will contribute to the growth of a Canada-wide database for all

eukaryotic life. Here, we present a combination of molecular, morphological and anatomical results for members of the genera *Pugetia* and *Euthora*. Currently, two species of *Pugetia* (*P. firma* & *P. fragilissima*) are recognized in B.C, however, our molecular results indicate *Pugetia* may have up to four taxa. As well, we have a group of samples field-identified as *Pugetia* for which molecular data, and subsequent anatomical examination, indicate no affiliation to the genera of the family Kallymeniaceae currently reported from B.C. We have resolved two species of the genus *Euthora* in B.C., where currently only one is recognized. The use of an integrated molecular, morphological, and anatomical approach to resolving taxonomic confusion within members of the family Kallymeniaceae in B.C. will be discussed.

13

GENE EXPRESSION IN PHLOROTANNIN-RICH TISSUE FROM THE BROWN ALGA *FUCUS VESICULOSUS*

Pelletreau, Karen N., Coyne, Kathryn J. & Targett, Nancy M.

College of Marine and Earth Studies, University of Delaware, Lewes, DE, USA

Phlorotannins are secondary metabolites unique to the Phaeophyceae. These compounds have many important ecological functions (e.g., anti-herbivore, UV protection, cell wall reinforcement), which have been widely investigated. Knowledge of the processes that underlie the production of phlorotannins is pivotal to comprehending their ecological function and importance because it enhances our understanding of cost/benefits and trade-offs involved in phlorotannin-mediated brown algal chemical ecology. The biochemical and molecular mechanisms at work in the synthesis of phlorotannins remain uncharacterized. Initial investigations by our lab utilizing biochemical mining, and directed approaches targeting candidate genes in brown algae generated no conclusive data. Subsequently, we generated a cDNA library using suppressive subtractive hybridization to isolate the genes that are over-expressed in algal tissue with constitutively higher phlorotannin content. The resulting sequence data provide evidence of several metabolic enzymes not yet described from brown algae, and suggest potential mechanisms involved in phlorotannin synthesis.

14

ECOLOGY OF THE FISH ASSOCIATED DINOFLAGELLATE *CREPIDOODINIUM CYPRINODONTUM*

Cooney, Sean K.^{1,3}, Stoecker, Diane K.² & Coats, D. Wayne³

¹*Marine, Estuarine, and Environmental Sciences Program, University of Maryland, College Park, MD, USA;*

²*University of Maryland, Center for Environmental Science, Cambridge, MD, USA;* ³*Smithsonian Environmental Research Center, Edgewater, MD, USA*

Crepidoodinium cyprinodontum is a photosynthetic dinoflagellate that lives on gill lamellae of fish. Known hosts for this dinoflagellate genus belong to the families Cyprinodontidae, Fundulidae, and Sillaginidae. *Crepidoodinium* has been considered by some investigators as a parasite and by others as a commensal. This uncertainty regarding the relationship of this dinoflagellate to its host stems from the complete lack of data about the organism's ecophysiology. We assessed the occurrence of *C. cyprinodontum* on cyprinodontid and fundulid species in Maryland and Florida waters. When present, *C. cyprinodontum* showed high prevalence, with epibiont load being highly variable among individual fish. Among host taxa examined, *F. majalis* exhibited highest epibiont prevalence and infection intensity. While prevalence on *F. majalis* was unrelated to host sex, load in males was found to be negatively correlated with host length. Comparison of load versus gill surface area indicate smaller hosts harbor higher epibiont densities in both sexes. The number of *Crepidoodinium* per host varied seasonally, with maximum values observed in summer months in Maryland waters. Differences in survival rates across a light gradient (relative to incident PAR) were detected, with higher rates observed at intermediate light levels. We also report two new host species, the longnose killifish (*F. similis* c.f.) and goldspotted killifish (*Floridichthys carpius*) from Florida waters.

15

SIGNIFICANCE OF DIATOM EPS IN FOOD WEBS OF COLNE ESTUARY BIOFILMS: TRACKING ¹³C THROUGH LIPIDS AND POLYSACCHARIDES TELLS THE STORY

Bellinger, Brent J.^{4,1}, Underwood, Graham J.², Ziegler, Susan E.³ & Gretz, Michael R.⁴

¹Soil and Water Science Department, University of Florida, West Palm Beach, FL, USA; ²Department of Biological Sciences, University of Essex, Colchester, United Kingdom; ³Department of Earth Sciences, Memorial University of Newfoundland, St. John's, NF, Canada; ⁴Department of Biological Sciences, Michigan Technological University, Houghton, MI, USA

The ubiquitous presence of algal exudates within biofilms from aquatic systems is well documented. However, the biochemical make-up and the specific role of polysaccharide rich extracellular polymeric substances (EPS) in mudflat biofilms has remained a black box due, in part, to the inherent complexity of the biofilms and lack of detailed information as to the makeup of the polymers. Utilizing an array of analytical techniques and applying them to uni-algal cultures and complex biofilms from the surface of intertidal mudflats within the Colne Estuary, U.K., we were able to characterize the structural properties of diatom EPS that provide insight into the central role of EPS functions in carbon flow dynamics within estuarine biofilms. The diatom species *Nitzschia epithemioides* and *Navicula phyllepta*, isolated from the Colne, produced EPS polymers with varying degrees of *O*-methylation and carboxylation, conveying greater or lesser hydrophobic and hydrophilic properties. The methylation patterns overlaid on the variable saccharide backbone, including carboxyl-containing units, results in differential persistence of EPS within biofilms subjected to tidal influences and in differential heterotrophic activity by bacteria. The role of EPS in the transfer of energy, as carbon, from fixation by diatoms to utilization by heterotrophic bacteria was elucidated utilizing carbon isotope pulse-chase labeling experiments. The largest amount of excess carbon was initially found within the diatom storage polymer chrysolaminaran and monosaccharides within EPS fractions. Chrysolaminaran was catabolized by diatoms for production of lipids and EPS over time. After numerous tidal cycles, a minimal amount of isotopically enriched diatom EPS remained, but the heterotrophic community (Proteobacteria and Firmicutes) within the sediments remained highly enriched. This work highlights the value derived from detailed biochemical studies of EPS in delineating the functional role of these polymers in estuarine systems, and the utility of combining multiple analytical techniques to clarify the ecological role of EPS within complex biofilms.

16

TAXONOMIC REASSESSMENT OF THE *CALOGLOSSA LEPRIEURII*-COMPLEX (DELESSERIACEAE, RHODOPHYTA), AND EXPERIMENTAL ELUCIDATION OF THE FUNCTION OF PIT CONNECTIONS

Krayesky, David M.¹, Norris, James², West, John³ & Fredericq, Suzanne¹

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The genus *Caloglossa* (Harvey) G. Martens (Delesseriaceae, Ceramiales) is an ecologically important intertidal component of mangroves and salt/freshwater marshes throughout tropical, subtropical and temperate regions worldwide. It is a small group in which less than twenty distinct species are known. Although the systematics of this genus has been intensely investigated, its diversity is not yet fully understood. One taxonomically confused group is the “*Caloglossa lepriurii*-complex”, recognized as a highly polymorphic taxon. On the basis of morphological, *rbcL* and 26S rDNA sequence data, the *C. lepriurii*-complex includes the generitype (described from French Guyana) and several distinct species. Two of these were found to be new species: one from the northern Gulf of Mexico and eastern USA, and another from the Florida Keys and Belize. These new species can be distinguished on the basis of vegetative characters: rhizoid morphology, degree of constriction at the thallus nodes, types of branching, cystocarp location, and number of cell rows cut off from the first axial cell of the main axis. While blade-like thalli of *Caloglossa* were known to contain abundant secondary pit connections that link kindred and non-kindred cells, the function of these pit connections among dorsal and lateral pericentral cells has been unclear. A combination of experiments using microinjection techniques and epifluorescence microscopy confirmed that these pit connections are involved in cell-to-cell communication.

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INSIGHTS INTO THE GROWTH AND FEEDING OF A TEMPERATE ISOLATE OF *MESODINIUM RUBRUM*

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The *Mesodinium rubrum* Lohmann 1908 (= *Myrionecta rubra* Jankowsky 1976) of this study holds a permanent cryptophyte symbiont with numerous chloroplasts, mitochondria, nucleomorphs and only one symbiont nucleus. The symbiont is delimited from the host by a single membrane and the chloroplasts are large and morphologically different from the *Teleaulax* species used for prey. *Mesodinium rubrum* is an obligate mixotrophic ciliate because it needs prey to uphold growth beyond 4 divisions. We have studied the functional and numerical response of *M. rubrum* under 2 light regimes (20 and 100 $\mu\text{E m}^{-2} \text{s}^{-1}$). *Mesodinium rubrum* needs a minimum of ~ 50 *Teleaulax* cells ml^{-1} to uphold a positive growth rate at both irradiances. Maximum growth rates were 0.23 and 0.49 d^{-1} at 20 and 100 $\mu\text{E m}^{-2} \text{s}^{-1}$, respectively. These growth rates correspond to ~ 1 *Teleaulax* cell *M. rubrum* $^{-1} \text{d}^{-1}$. The maximum ingestion rates were independent of light irradiance and a maximum ingestion rate of ~ 6 *Teleaulax* cell *M. rubrum* $^{-1} \text{d}^{-1}$ was observed. An ingestion corresponding to only 2-4% of the carbon content of *M. rubrum* was needed to uphold maximum growth rate, but a carbon contribution of $\sim 22\%$ was observed with no additional effect on growth rates. The implications of elevated pH levels in the cultures proved to be substantial as the growth of *M. rubrum* and *Teleaulax* sp. was impeded at pH levels in excess of 8.5 and 8.8, respectively. Starvation experiments showed that *M. rubrum* cultures are capable of enduring ~ 50 days with no prey before they succumb. These results will be included in a discussion of the propagation of *Mesodinium rubrum* in time and space.

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DO PLANKTONIC EURYHALINE PROTISTS EXIST?

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The diversity of aquatic protists is beginning to be appreciated both in terms of cultured organisms and by way of environmental genetic surveys notably using the 18S rRNA gene. Historically there have been many species that have been reported from both freshwater and marine habitats. Recently molecular surveys of cultured morphospecies have suggested that at least among some groups, morphospecies are widely divergent and contain cryptic stenohaline species. Our results from the Mackenzie River, its estuary and adjacent Beaufort Sea (Arctic, Canada) suggested the predominance of stenohaline protists. The diversity of protist sequences in the Mackenzie River was particularly striking and provided a core data set used to compare with other sequences in publicly available data bases.

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ACANTHAMOEBA DISTRIBUTION IN THE CHESTER RIVER ON THE EASTERN SHORE OF MARYLAND

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The large species diversity of amoebae in soils and sewage sludge reported by Singh and Hanumai (Monograph No. 1 of the Association of Microbiologists of India, Indian Journal of Microbiology, 1979) was found to serve as a useful indicator of soil erosion and sewage pollution in soils and aquatic sediments. *Acanthamoeba* is a ubiquitous soil amoeba that is often associated with sewage pollution. Certain species of the genus are causative agents of human disease (GAE and keratitis). The Chester River is a tributary to the Chesapeake Bay on Maryland's eastern shore. The river, although not highly polluted, is somewhat adversely impacted by two factors, sewage pollution and nutrient runoff from agricultural lands. Sediments from 11 sites along the river were collected and cultured for the presence of *Acanthamoeba*. All sites yielded amoebae, and most harbored potentially pathogenic, temperature tolerant (39C) species. The most commonly isolated species were *A. polyphaga*, *A. rhyssodes*, *A. hatchetti* and several other unidentified isolates that belonged to either Group II or Group III of the genus. Certain species appeared to be more associated with sewage pollution.

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AMOEBAE FROM SALINE ENVIRONMENTS HARBOR *LEGIONELLA* SPECIES

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Amoeba cultures were recovered from sediments from the New England estuarine system of Mt Hope Bay and from the Great Salt Lake. Sampling sites in Mt Hope Bay included the thermal plume region of a power plant, a secondary sewage outfall, a brackish environment and a coastal marine region considered impacted by normal bay conditions. The sites in the Great Salt Lake included a canal used by sewage treatment plants to release water into Farmington Bay, areas near the opening in the causeway that separates the Farmington Bay from Gilbert Bay, the bathing beach in Bridger Bay (in Gilbert Bay) and in Bear River Bay. Enrichment for amoebae was accomplished using both minimal and non-nutrient agar plates, made with fresh water, brackish water or saltwater. The amoeba cultures that were recovered were assayed for the presence of *Legionella* species using nested PCR and primers specific for the genus. Positive samples were then screened with nested primers specific for *L. pneumophila*. Fiftytwo of 90 isolated amoeba cultures were positive for the presence of *Legionella* species. *L. pneumophila* was detected by PCR in ten of the amoeba cultures growing on marine media. Our results show that amoebae capable of growing in saline environments harbor not only a diverse collection of *Legionella* species, but also species pathogenic to humans.

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THE NON-PATHOGENIC X-BACTERIA (*CANDIDATUS LEGIONELLA JEONII*) IN SYMBIOSIS IN *AMOEBA PROTEUS* HAVE VARIATIONS IN PATHOGENICITY GENES IN A COMPARATIVE GENOMIC ANALYSIS

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The X-bacteria, an obligate endosymbiont in *A. proteus* is a temperature sensitive non-pathogenic *Legionella* sp. They infect amoebae through phagocytic pathway as pathogenic *L. pneumophila* but exert no harm to the host. To understand the non-pathogenic characteristics of X-bacteria we carried out a comparative genomic analysis using *L. pneumophila* as a reference organism. To date a total of 692 non-redundant clones of genomic DNA library of X-bacteria in lambda ZAP were tagged by nucleotide sequencing. Among 1399 genes detected in 1,183 Kb we identified 691 genes of assigned functions and 328 conserved hypothetical genes of high similarity and 389 genes of no similarity. Among high homologous genes, 585 genes (52.%) had the best matches in *L. pneumophila* database. We obtained 53 complete ORFs (av. pl 5.17) and detected 34 genes related with infection and endocellular life style, including genes for virulence (*dotA*, *icmKTSV*, and *lvrABC*), infection and pathogenicity (*rmp*, *rhuM*, *rpfB*, and *sbpA*), drug resistance (*mdfA* and *norM*), and type IV secretion system (*virB4*). In addition, we detected 28 putative transposases relevant to genetic interaction among genomes in the symbiotic system. In comparisons of physico-chemical properties, pathogenicity genes, *icmTS*, *icmK*, and *icmV* showed similarity to those of *L. pneumophila*. In comparisons of 5 *icm/dot* components for Type IV secretion system, the amino acid sequences of each gene were relatively well conserved. However, *dotA* was disrupted by *icmV*, and *icmTS* and *icmK* genes were translocated among non-pathogenic genes. These results imply that the lifestyle of the symbiotic *L. jeonii* in amoebae is different from that of *L. pneumophila* in human. Thus, the symbiotic *L. jeonii* may be a good reference organism in the study of pathogenicity of *L. pneumophila* in human.

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A RECENT PLASTID ESTABLISHMENT IN THE THECATE AMOEBIA *PAULINELLA CHROMATOPHORA*

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A critical event in the evolutionary history of eukaryotes was the establishment of plastids (e.g., chloroplasts). The origin of the first plastid can be traced to a single primary endosymbiosis where a non-photosynthetic protist engulfed and enslaved a cyanobacterium as a cytoplasmic organelle. Plastids containing chlorophyll

were capable of carrying out photosynthesis. Over time, plastids allowed for the evolution of algae and the plants, which form the base of the food chain for life on modern earth. Descendants of this first primary endosymbiosis gave rise to the glaucophyte, green and red algae. By secondary and tertiary endosymbioses, photosynthesis spread and gave rise to more eukaryotic groups (e.g., brown seaweeds, diatoms and dinoflagellates). However, our knowledge of plastid evolution is still limited because those endosymbiotic events occurred more than 1.5 billion years ago. Here we analyze the plastid genome in *Paulinella chromatophora*, a thecate amoeba that contains the only known case of a recent independent primary plastid acquisition. Single and multi-gene phylogenetic analyses demonstrate a close, sister group relationship of the *Paulinella* plastid with *Synechococcus* sp. WH5701. Using three regions of the plastid genome (ca. 19,000 bp), our analysis shows that the gene order for *P. chromatophora* is nearly identical to that found in the *Prochlorococcus-Synechococcus* clade of cyanobacteria. These data demonstrate a recent plastid capture by *P. chromatophora*, and they suggest that the complete plastid genome is likely to be of cyanobacterial proportions rather than the much smaller size found in plastid genomes. Therefore, because the *P. chromatophora* cyanelle genome has not undergone an obvious size reduction, important insights to the early stages of cyanobacterial endosymbiosis and plastid establishment are likely to be present in *P. chromatophora*. We suggest that key processes, such as the control of organelle division and translocation of fixed carbon across the cyanelle membranes to the host, will be learned by studying the *Paulinella* nuclear genome rather than its endosymbiont.

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SYMBIONT HETEROGENEITY IN REEF FORAMINIFERA

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Several genera of foraminifera host *Symbiodinium* dinoflagellates, the same symbionts found in reef-building corals and many other invertebrates. While much recent work has been done to examine the distribution and biogeography of these symbionts among their host lineages of foraminifera, little has been done to examine the variation of *Symbiodinium* within host individuals. By cloning amplified *Symbiodinium* rRNA sequences, I show that there is appreciable genetic heterogeneity to symbionts within individual *Amphisorus hemprichii* foraminifera from Papua New Guinea. Moreover, different symbiont types are found within different parts of an individual host. The data suggest that this distribution may be related to sorting of newly encountered symbionts from the environment, and raises important questions about the mechanism of symbiont recognition in foraminifera.

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PLASTID RETENTION AND FUNCTIONALITY IN THE DINOFLAGELLATES *DINOPHYSIS ACUMINATA* AND *DINOPHYSIS CAUDATA*

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Dinophysis acuminata and *D. caudata* must feed upon the plastidic ciliate *Myrionecta rubra* to remain viable. Whether or not *Dinophysis* has its own permanent chloroplasts or retains plastids from its prey, however, remains unresolved. Using cultures of *Dinophysis*, we examined if *Dinophysis* plastids are derived from *M. rubra*, and further how long *Dinophysis* plastids (or kleptoplastids) persist and remain photosynthetically active in the absence of prey. Stock cultures of *D. acuminata* and *D. caudata* grown in f/2 medium were fed *M. rubra* daily and maintained at 20°C under fluorescent lamps providing 60 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. Stock cultures were washed free of prey and sampled at 3-7 day intervals over > 2 months to assess cell numbers, in vivo fluorescence before and after addition of DCMU, cellular starch content, presence of acid vacuoles, and the status of the plastid 16S rRNA gene and psbA gene. While *D. acuminata* and *D. caudata* plastids persisted up to more than 2 months, their photosynthetic activity was quickly diminished and was lost within 1 month. Thereafter, the plastids began to be digested. Our results confirm that the chloroplasts of *D. acuminata* and *D. caudata* are kleptoplasts, and indicate that the *Dinophysis* species are primarily heterotrophic, but use autotrophy through kleptoplastidy as a complementary or short-term survival strategy.

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A DAY IN THE LIFE OF THE ZOOXANTHELLATE CILIATE *MARISTENTOR DINOFERUS*: A DISPERSAL RHYTHM AND BEHAVIORAL DEFENSES AGAINST GRAZING FISH

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Zooxanthellate ciliates are unusual *Symbiodinium* hosts in being mobile; this allows behavioral responses to their environment. *Maristentor* is unusual among the few ciliates in this group in being observable in the field. It forms visible clusters on a variety of coral reef substrata. Populations are patchy on several spatial and temporal scales. The substrata are dynamic: microbial films develop and are eaten by fish, sediment settles and is sometimes swept away, and some substrata (notably *Padina*) are living and growing. The clusters are likewise dynamic groups of basally attached individuals that change hourly and daily, within larger patches that are persistent over weeks, even in areas with intense grazer activity. In the field the daily dispersal/clustering pattern starts with almost all individuals in clusters at the end of the night. Starting at sunrise and taking about 2 h, the clusters disperse as more and more individuals glide away from them, and about an hour later cells begin to cluster again. With sunrise at 0600h clusters are clearly visible again by 1000h, even though many cells are still gliding around individually. By noon most cells are in clusters, but clusters become larger and fewer in a given area throughout the afternoon as individual cells migrate (“cocktail party dynamics”) and some clusters disperse abruptly. Grazing fish (especially *Ctenochaetus striatus*) remove patches of microbial film but appear to avoid *Maristentor* clusters. *Maristentor* pigment has been chemically characterized and is almost certainly toxic, and the black spots appear to act as a visual warning to fish. Grazing pressure is lower during the hours when the clusters are dispersed. *Maristentor* accumulates preferentially on bare areas, including recently grazed areas and vertical surfaces, which act as refugia.

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PHOTOTROPHIC *MYRIONECTA RUBRA* IS A REMARKABLE “MARINE LINKING CILIATE”

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Based on the first observation of FLB (fluorescently labeled bacteria) ingestion by the cells of MR-MAL01, a temperate strain (GenBank accession number: EF195734) of the photosynthetic ciliate *Myrionecta rubra* Jankowski 1976 (= *Mesodinium rubrum* Lohmann 1908), *M. rubra* has been recognized as a marine bacterivore (Myung et al. 2006). The ingestion rates of the *M. rubra* in cultures (ca. 1.0×10^4 cells ml⁻¹) were calculated to be 53 bacteria grazer⁻¹ h⁻¹, and the bacterivory rate of *M. rubra* increased gradually as light intensity decreased from 200 down to 0 $\mu\text{E m}^{-2} \text{s}^{-1}$: Given 10×10^6 bacteria ml⁻¹, each *M. rubra* cell ingested 159, 97, and 71 bacteria h⁻¹ at 0, 60, and 200 $\mu\text{E m}^{-2} \text{s}^{-1}$, respectively. Natural populations of *M. rubra* in Korean coastal waters (Masan Bay, Keum River Estuary, and Saemankeum Lake) also ingested FLB to exhibit bacterivory at rates which were comparable to the total bacterivory by all the co-existing HNF. Even though the measured *in-situ* ingestion rates of the *M. rubra* populations (2-17 bacteria grazer⁻¹ h⁻¹) was not greater than that of the temperate strain MR-MAL01 (i.e., 53 bacteria grazer⁻¹ h⁻¹), *M. rubra* may sometimes exert greater impact on the bacterial community (daily removal of 0.2-27.8%) than total co-existing HNF (1.0-18.8% d⁻¹). Considering the cosmopolitan occurrence and frequent blooms of *M. rubra* populations in diverse coastal and eutrophicated off-shore waters, here we suggest that *M. rubra* should be another remarkable “linker” between marine microbial loop and metazoan food web (Sherr & Sherr 1987, Sorokin et al. 1999). 1. Myung G, Yih W, Kim HS, Park JS, Cho BC (2006) Ingestion of bacterial cells by the marine photosynthetic ciliate *Myrionecta rubra*. *Aquat Microb Ecol.* 44, 175-180. 2. Sorokin YI, Sorokin PY, Ravagnan G (1999) Analysis of lagoonal ecosystems in the Po River Delta associated with intensive aquaculture. *Estuar Coast Shelf Sci.* 48, 325-341. 3. Sherr EB, Sherr BF (1987) High rates of consumption of bacteria by pelagic ciliates. *Nature* 325, 710-711.

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EOL - BUILDING BRIDGES BETWEEN BARCODES AND TRADITIONAL INFORMATION

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Barcoding offers a prospect of rapid identification of clades of life. The process is a computerized. How can we extend the pipeline to reach out beyond the name of the clade to show us pictures, tell us where it has been found, who has published recently on it, and whether it represents any kind of threat to public health or the ecosystem. The Encyclopedia of Life (<http://www.eol.org>), funded by the MacArthur and Sloan Foundations, has the task of delivering a web page for all named species within 10 years. The foundations of this vision are being established by the Smithsonian Institution, Harvard University, the Field Museum, the Biodiversity Heritage Library, the MBL in Woods Hole, and the Missouri Botanical Garden. The project uses 'Taxonomic Intelligence' to embed the best elements of taxonomic practices in the management of biological data; aggregation (mashup) technologies to draw distributed data together, Web 2.0 thinking to enable community participation, and to provide the semantic framework that will vastly increase the utility of EOL. EOL presumes that most information about organisms will be accessible on the internet within 10 years. The EOL project will promote the emergence of collaborative tools that can gather the information together and to collate it to create web pages. In one sense, barcodes are metadata, akin to and interoperable with names, that can be used to annotate data about organisms. EOL can assist the integration of molecular discovery and auditing tools with traditional insights. Assuming that barcodes will be seamlessly integrated within the EOL, one of the other goals must be the e-mobilization of the data into a form that EOL can exploit. ISOP has an opportunity to play a singular coordinating role in this process.

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CHLOROPLAST EVOLUTION: PAST AND PRESENT

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There is general agreement that present day chloroplasts are derived from once free-living cyanobacteria. Furthermore the extant chloroplast lineage(s) is based on the assumption that an autotrophic cyanobacterium entered a eukaryotic host and via endosymbiotic events formed a permanent stable association. The high structural/functional similarities of the photosynthetic reaction centers (I and II) provides the most cogent support for this view. It also assumes that such an association would not have caused oxygenic stress produced from photosystem II. Essential factors in facilitating a stable association between the endosymbiont and host are: (a) retention/transfer of genetic material between, (b) import and export of proteins, (c) allocation of carbon and nitrogen metabolites, (d) co-ordination of isoprenoid biosynthesis, and (e) selective pressure(s). The sequencing of the *Porphyra purpurea* genome (S. Brawley, et al.) by JGI will potentially provide an important basis for furthering the understanding of chloroplast evolution.

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THE CYANELLE OF *CYANOPHORA PARADOXA*: MISSING LINK OF PLASTID EVOLUTION

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Glaucocestophyte algae represent the primordial phototrophic eukaryotes on the first branch after the single primary endosymbiotic event between a heterotrophic protist and a cyanobacterium. Their plastids (cyanelles) are the closest cousins to free-living cyanobacteria. The position of *Cyanophora paradoxa* as bridge organism and "living fossil" is underlined by unique prokaryotic features of cyanelles shared by no other plastid type: the peptidoglycan wall surrounding the organelle and (likely) the carboxysomal nature of the Rubisco microcompartment involved in the carbon-concentrating mechanism. Isolated cyanelles can be treated as "honorary cyanobacteria" and processes as phycobilisome and carboxysome assembly can be studied after import of labelled precursor proteins and subsequent fractionation. Dual Sec translocases exist in the cyanelle thylakoid and inner envelope membranes as found in cyanobacteria but in contrast to chloroplasts. The protein import apparatus of cyanelles, though a "prototype", nevertheless is homologous to the Toc-Tic translocases of higher plant chloroplasts. This is essential when a single primary endosymbiotic event is assumed and was corroborated through heterologous *in vitro* import experiments in both directions.

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PLASTID KLEPTOPLASTY: ONE FIRST STEP TOWARD ENDOSYMBIOSIS

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Elysia chlorotica is a sacoglossan mollusc (sea slug) that feeds on the heterokont alga *Vaucheria litorea*, and retains the algal chloroplasts in a functional kleptoplastic association for several months. No algal nuclei have been detected within the mollusc and the *V. litorea* chloroplast genome only encodes 139 proteins; this is far fewer than the expected minimum of 2000 proteins needed to sustain chloroplast activity. This begs one to ask how oxygenic photosynthesis, linked to electron transport, can be maintained in the mollusc without the necessary proteins encoded in the genes of the algal nucleus. Our current studies are focused on demonstrating the presence of algal nuclear genes in the sea slug as a result of horizontal gene transfer (HGT). Employing PCR, we identified two nuclear encoded, essential chloroplast proteins that are not found in any animals, specifically, PRK (phosphoribulokinase) and PsbO (Mn-stabilizing protein of the oxygen evolving PSII complex). Two copies of *prk* were found in the sea slug, each containing the nucleotide region spanning exon 1 and part of exon 2 of *V. litorea prk*, including the bipartite chloroplast transit peptide. However, the larger *prk* fragment possesses intron 1 while the smaller fragment is intronless. Both *prk* copies were detected in adult animals containing kleptoplasts, as well as in plastid-free eggs produced by the sea slugs in culture. PCR, RT-PCR and Northern-blotting were used to demonstrate the presence and expression of *psbO* in the sea slug. Sequencing of the PCR products revealed high similarity for *psbO* sequences from genomic DNA and cDNA of the sea slug and alga. As expected, neither of these nuclear encoded genes is found in the chloroplast genome of *V. litorea*. Hence, we propose that gene transfer has occurred from *V. litorea* to the nuclear genome of *E. chlorotica* which allows the mollusc to maintain photosynthesis. Genome walking is being employed to examine the extent and mechanism of DNA transfer between the alga and sea slug. We hope to develop an increased understanding of HGT between these two multicellular eukaryotes and the evolution of kleptoplasty and photosynthesis in a mollusc.

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GIARDAN: STRUCTURE, SYNTHESIS, REGULATION AND INHIBITION

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During encystment, *Giardia* trophozoites become encased in a filamentous extracellular matrix that consists of a novel 2-acetamido-2-deoxy-D-galactan we are naming giardan. Giardan is synthesized from glucose via sugar phosphate intermediates to UDP-GalNAc by inducible, cytosolic enzymes. The UDP-GalNAc is fixed into giardan apparently by an inducible, particle-associated transferase. Regulation of this synthesis appears to center around pyrophosphorylase, epimerase and cyst wall synthase (Cws) activities. Pyrophosphorylase seem to be involved in making sufficient UDP-N-acetylglucosamine (GlcNAc) to drive the epimerase kinetics toward UDP-GalNAc synthesis while the Cws removes intracellular UDP-GalNAc extruding it as giardan and thus preventing an increased intracellular concentration of UDP-GalNAc that could drive the reaction toward GlcNAc synthesis. Encystment is usually caused by depletion of a vital nutrient and assuming encystment is irreversible at some point, then inhibiting encystment, especially late in the process, could cause the encysting trophozoites to die rather than just stop encysting. While this concept has yet to be proven experimentally, it does seem likely that there exist targets in the pathways for potential drug design. Two likely targets are epimerase (Uae) and Cws since both are at the end of the encystment synthetic pathway when the cell is most likely committed. *Giardia's* Uae differs from the human Uae, and Cws most likely is not found in humans or any mammal. Unfortunately few inhibitors of these enzymes are known. A 2-amino-2-deoxyglucitol-6-phosphate, a GlcN-6-P analogue, inhibited the activity of glucosamine 6-phosphate deaminase while Jarroll (unpublished) observed that this same analogue at 1mM reduced encystment in vitro from ~70% to ~2-3%. Inhibition by this analogue in vitro did not appear to cause trophozoite death during the four day period for which it was observed. Cws requires a divalent cation and EDTA, a chelating agent, inhibits Cws activity in vitro at 1 mM or higher. Studies are currently underway to determine if an echinocandin, a beta (1, 3) glucan synthase inhibitor for fungi, will inhibit Cws.

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THE 'HYDROGENOSOME' AT THIRTY-FOUR YEARS OF AGE AND ITS ROLE IN THE ACTIVITY OF METRONIDAZOLE

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Hydrogenosomes were first described, characterized and named by Lindmark and Muller in the parabasilid flagellate, *Tritrichomonas foetus*. The organelles are 1-2 μm in size and compartmentalize the terminal reactions of anaerobic cellular energy metabolism. The Hydrogenosome is found in other trichomonads, some anaerobic rumen ciliates, termite flagellates and Fungi. In recent years, this organelle has been investigated as to its evolutionary significance and metabolic properties. In this presentation, data are presented on early and current research in both areas. The Hydrogenosome contains pyruvate ferredoxin oxido-reductase (PFOR). PFOR is found in other anaerobic microbes (*Giardia*, *Hexamita*, *Entamoeba* and anaerobic bacteria, *Clostridium* and *Bacteroides*) lacking hydrogenosomes. This enzyme was shown by Lindmark and Muller to be involved in the conversion of metronidazole into a toxic compound, in studies with *Trichomonas*, *Tritrichomonas*, *Giardia* and *Entamoeba*. Subsequent studies demonstrate that is the same with anaerobic bacteria, hence, metronidazole is now recognized as an important chemotherapeutic agent ('gold standard') in the treatment of anaerobic infections. This presentation will discuss the recent data on the action and current use of metronidazole.

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ATP BINDING CASSETTE (ABC) TRANSPORTERS AND DRUG EFFICACY IN *CRYPTOSPORIDIUM PARVUM*

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Cryptosporidium parvum is a parasite that has demonstrated a lack of susceptibility to chemotherapeutic agents. Several ATP-binding cassette (ABC) transporters have been identified in the protozoan parasite *C. parvum*, but little is known about their function or possible role in drug efflux. These pumps probably contribute to protecting the parasite from toxins, including chemotherapeutic agents. Evidence for the phenotypic expression of these ATP-binding transporter pumps was previously demonstrated by the decreased calcein uptake by parasites in culture after depletion of the available ATP with 2-deoxyglucose and by treatment with probenecid. Naringenin, a flavonoid compound that binds to the nucleotide binding (NB) site of *Leishmania* p-glycoprotein, decreased calcein uptake in *C. parvum* compared to controls when tested at 40 μM . Flavonoids were subsequently tested alone and in combination with potential anticryptosporidial compounds. Naringenin and genistein were most active with EC_{50} of 15 μM and 25 μM , respectively. The EC_{50} of trifluralin was decreased significantly when combined with genistein in an in vitro assay, suggesting that flavonoid compounds may be used alone or in combination with other moderately active drugs to increase anti-parasite efficacy. In addition, we studied three *C. parvum* ABC transporters (cgd1_1350, cgd7_4510 and cgd7_4520) of *Cryptosporidium parvum* identified with high homology to nucleotide binding domains of other parasite NB domains. To determine if drug treatment modulated the 3 transporters, infected HCT-8 cells were incubated with different concentrations of paromomycin and a single concentration of cyclosporin A. Our results indicated paromomycin treatment upregulated RNA transcript levels of cgd1_1350 by ~5 to 8 fold while cgd7_4510 levels rose ~3 fold. Cyclosporin A had a similar upregulating effect on cgd1_1350 and cgd7_4510 RNA levels: 5 fold and 2.6 fold, respectively. On the contrary, drug treatment had little effect on cgd7_4520 transcript levels. The intrinsic drug resistance in *C. parvum* may be overcome by rational drug design and by understanding and exploiting the transporters involved in drug uptake and efflux.

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NOVEL COMPARTMENTALIZATION OF *CRYPTOSPORIDIUM PARVUM* PYRUVATE:NADP⁺ OXIDOREDUCTASE WITHIN THE CRYSTALLOID BODY

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Most eukaryotes perform the oxidative decarboxylation of pyruvate within mitochondria using pyruvate dehydrogenase (PDH). Anaerobic parasitic protists without mitochondria lack PDH, using instead the O₂-sensitive enzyme pyruvate:ferredoxin oxidoreductase (PFO). Unlike most of these protists, both *Cryptosporidium parvum* and *Euglena gracilis* encode and express a unique O₂-sensitive enzyme, pyruvate:NADP⁺ oxidoreductase (PNO). Analysis of *C. parvum* PNO (CpPNO) revealed that the cDNA encodes an N-terminal PFO domain that is fused to a C-terminal NADPH-cytochrome P450 reductase (CPR) domain. Unlike *E. gracilis*, the PNO of *C. parvum* lacks a mitochondrial targeting peptide. However, *C. parvum* does possess a small “relic mitochondrion” sandwiched between the nucleus and crystalloid body (CB). Recent cryo-EM and tomography reveal possible direct connections between the nucleus and relic organelle. The atypical “cristae” within the relic mitochondrion are confirmed, and the membrane-bounded vesicles of the CB appear to be wrapped by a filamentous matrix. Using polyclonal antibodies against both the CpPFO and CpCPR domains of the fusion protein, both confocal immunofluorescence and immunogold-EM confirmed that, as expected, CpPNO did not target the relic mitochondrion. Surprisingly, CpPNO occupied two distinct sporozoite compartments: the cytosol and CB. We are currently isolating both the relic mitochondrion and CB in order to explore by proteomics whether one or both of these highly specialized organelles is involved in *C. parvum* core metabolism, and/or the biosynthesis of iron sulfur clusters, a essential function of eukaryotic mitochondria. In summary, we have shown that unlike a similar fusion protein in *E. gracilis*, CpPNO targets the cytosol and CB, not the relic mitochondrion. Because this protein appears to be compartmentalized in a novel way, it opens the interesting possibility that CpPNO represents yet another unique type of core energy metabolism in anaerobic protists. It also suggests that this unusual compartmentalization might lead to new strategies for drug development against human cryptosporidiosis.

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EFFECTS OF ION-CHANNEL BLOCKERS ON *PLASMODIUM FALCIPARUM* VIABILITY AND THEIR POTENTIAL USE AS ANTI-MALARIAL TARGETS.

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Plasmodium falciparum is the causative agent of human malaria, a disease responsible for between 2-3 million deaths per year. Acidic organelles have been described in a variety of *Plasmodium* and trypanosomatids. The *Plasmodium* acidic food vacuole is involved in hemoglobin digestion and ion homeostasis, as well as being the target for a number of antimalarial drugs such as chloroquine (CQ). Manipulation of parasite ion physiology results in significant effects on parasite viability, CQ uptake, and CQ susceptibility. For example, experimental manipulations of Cl⁻-dependent ion homeostasis (changing [Cl⁻]_o or using specific ion channel blockers) significantly and differentially alters parasite viability and CQ pharmacology. Parasite viability decreases with lower external chloride ([Cl⁻]_o), with the CQ resistant strains being less susceptible to loss of [Cl⁻]_o. CQ uptake and susceptibility (IC₅₀) are also differentially altered between strains as a function of [Cl⁻]_o. The most significant result was obtained with the stilbene DIDS, a blocker of several types of chloride channels and exchangers, including the cellular pH regulator Anion Exchanger (AE). Significant effects were also observed with other membrane-active compound such as nigericin and amiloride. Our results support the idea that alterations in ion-based physiologic parameters can be exploited as alternative targets for chemotherapy.

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ACROSS SPACE AND OVER TIME: PROCESSES THAT INFLUENCE POPULATION GENETIC STRUCTURE OF *FUCUS VESICULOSUS* L. IN THE NORTHWESTERN ATLANTIC

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The genetic structure of marine species in the northwestern Atlantic must be evaluated in the context of contemporary processes (e.g. hydrodynamics) as well as past events (e.g. the last glacial maximum, 19.5 - 15 kyr BP). We analyzed population genetic structure of the marine alga *Fucus vesiculosus* L. (Phaeophyceae) in the northwestern Atlantic using microsatellite markers to investigate the processes that determine genetic structure at small (i.e. around a coastal point, 1 m - 20 km) and large (i.e. the northwestern Atlantic, 1000s of kilometers) geographic scales. Reproduction in *F. vesiculosus* is confined to calm, sunny conditions and low dispersal capacities of gametes and zygotes suggest spatial genetic structuring. Using surface drifters, we characterized nearshore circulation patterns around study sites to investigate whether gene flow correlated with currents. We examined the longevity of *F. vesiculosus* eggs and sperm and viability of zygotes produced from crosses with aged gametes to evaluate how life-history stages contribute to gene flow. There was significant genetic differentiation at the two coastal promontories studied, but patterns of differentiation were complex and did not have within-site spatial structure. Our genetic and nearshore circulation data, combined with our gamete longevity study, supports the dependency of gene flow on storm-detached, rafting adults. At larger geographic scales, we found distinctive northern and southern groups in the northwestern Atlantic. There was significant genetic differentiation, unexpected patterns of genetic diversity, and a pattern of isolation by distance among the locations studied. Populations of *F. vesiculosus* at its southernmost boundary (Beaufort, NC) lack genetic diversity, and examination of herbarium specimens reveal that the contemporaneous low genetic diversity has existed at least 40 years. These studies highlight the significance of rafting as a mechanism for structuring established populations of macroalgae and associated biota at both small and large geographic scales (Supported by NSF OCE-99043, PSA Grant-In-Aid of Research).

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AXENIC CULTIVATION OF THE HETEROTROPHIC DINOFLAGELLATE *PFIESTERIA SHUMWAYAE* AND OBSERVATIONS ON FEEDING BEHAVIOR

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Heterotrophic dinoflagellates are widespread in aquatic ecosystems and have been increasingly recognized as important components of aquatic food webs. Knowledge of heterotrophic dinoflagellate feeding behavior and nutritional requirements is essential for assessing the role and impact of this group of protists on microbial communities. Most heterotrophic dinoflagellates are phagotrophs, and laboratory cultures generally are maintained with live prey. Research on cellular physiology and nutrition often requires complete control of experimental variables and can be difficult to conduct with cultures containing multiple species. Axenic cultivation, the growth of a species in the absence of other metabolizing cells, allows examination of cellular processes without potentially confounding interactions with other living organisms. Further, axenic cultivation is the most direct approach for defining and quantifying, in biochemical terms, the nutritional requirements of a species. Despite the importance of heterotrophic dinoflagellates in aquatic ecosystems, their nutritional requirements are not well known, and few species have been cultured axenically. In the present research, an undefined, biphasic culture medium was formulated that supported the axenic growth of two strains of the heterotrophic dinoflagellate *Pfiesteria shumwayae*. Dinoflagellate cells were observed ingesting particles in the biphasic medium, allowing detailed observations of feeding behavior. This research represents an initial step toward a defined axenic culture medium and subsequent determination of *P. shumwayae* nutritional requirements.

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UNDERSTANDING DINOFLAGELLATE GENOME: INSIGHTS FROM GENOMIC DATA AND PCNA CHARACTERISTICS

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Dinoflagellates are unicellular algae (heterotrophic protists for those without a chloroplast), ecologically important but “unusual” in cytology, genetics, and evolution. Recognized dinoflagellate genomes contain 2~280 pg DNA/cell (nearly 1~100 fold that of human haploid genome). The wide range of genome size appears to be correlated with cell size variation and partially account for variation in growth rate. All these heritable traits are likely the outcomes of genome remodeling and genome size evolution under variety of environmental forces throughout the evolutionary history. Thus dinoflagellates are excellent models for eukaryotic genome evolution and comparative genomic studies. We are tackling dinoflagellate genome evolution by analyzing genome features. From dinoflagellate EST data, we found dinoflagellate genes have relatively high GC content (50-60%). The most updated genome sequence data for prokaryotes, eukaryotes, organelles, and viruses were collected and the relationships between genome size and gene number as well as per cent coding were measured. Based on the correlations and known genome size, dinoflagellate genome was estimated to contain 38-280 thousand genes. Despite the apparent high number of genes, the coding region would only be a minute part of the genome (<2.4%). We further investigated gene copy number of the nuclear gene proliferating cell nuclear antigen (PCNA) in dinoflagellates. PCNA gene copy number appears to correlate with genome size, suggesting that dinoflagellate genome has undergone successive duplication in evolution.

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EFFECTS OF NUTRIENT LIMITATION AND SALINITY STRESS ON GROWTH AND PHOTOSYNTHESIS IN THE GREEN ALGA *PICOCHLORUM OKLAHOMENSIS*

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The effects of bicarbonate, phosphate and iron limitation and salinity stress on growth and photosynthesis were examined in the green alga *Picochlorum oklahomensis*. Algal cells were grown in batch cultures under either nutrient sufficiency (control) or nutrient limitation at salinities of 10, 50 and 100 ppt. Generally, nutrient limitation and increasing salinity from 10 to 100 ppt resulted in significantly lower cell yields and growth rates. Relative to high nutrient controls, P_{max} per cell was lower in low phosphate and low iron media but higher in low bicarbonate at 10 and 50 ppt, though not statistically significant. Chlorophyll content decreased in cells grown in nutrient-limited media. Iron deficiency caused major decreases in chlorophyll, reduced chl *a/b* ratio at 50 ppt and in the efficiency of photosystem II photochemistry as indicated by the ratio of variable to maximum fluorescence (F_v/F_m).

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MORPHOMETRIC, REPRODUCTIVE AND MOLECULAR CHARACTERIZATION OF *PSEUDO-NITZSCHIA DELICATISSIMA* (CLEVE) HEIDEN

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A cosmopolitan diatom species, *Pseudo-nitzschia delicatissima* has been shown to be intermittently toxic. Some strains from Canada, and New Zealand have been found to produce domoic acid, whereas other strains isolated from the same locations have not had measurable levels of toxins detected. It has been shown that semi-cryptic diversity is prevalent within this “species”. Although very little morphological variation is noted between strains in cultures around the world, molecular analyses, as well as mating attempts have shown that there are more than one species within the *P. delicatissima* complex worldwide. Approximately 50 clones morphologically consistent with the definition of *P. delicatissima* have been isolated into culture from waters in eastern Canada, including the Bay of Fundy, Bedford Basin and the Northumberland Strait. Through mating experiments the sex of 12 clones has been determined, 6 which are male, and 6 female. HPLC analyses were completed to determine whether any domoic acid producers were present among these clones. All were found

to be non-toxic. The ITS region of the ribosomal DNA is being amplified, sequenced and aligned to determine whether there are differences between clones that mate successfully, and those that do not. If differences are found, further investigations will follow on the potential for mating compatibility. Mating results, sequence data and discrete morphological differences may suggest the existence of semi-cryptic diversity in the *P. delicatissima* complex in Eastern Canadian coastal waters.

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ACTION OF DINITROANILINE HERBICIDES IN THE PROTOZOAN PARASITE *TOXOPLASMA GONDII*

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Toxoplasma parasites and relatives like *Plasmodia* are sensitive to dinitroaniline herbicides. It is hypothesized that dinitroanilines disrupt parasite microtubules. Our lab and others have demonstrated the effect of dinitroanilines on *Toxoplasma* replication -- parasites grow, replicate their DNA, but cannot split apart causing parasites to take on a blobbed appearance. Studies on herbicide resistance in goosegrass and green algae identified point mutations in α -tubulin genes correlated with resistance to dinitroanilines. Through chemical mutagenesis, we have generated *Toxoplasma* parasite clones resistant to 1 μ M benfluralin or trifluralin herbicides. Parasites resistant to benfluralin exhibited a point mutation in nucleotide position 1801 of α -tubulin (guanine to adenine substitution) which, when translated, revealed a substitution of valine with methionine at amino acid 252. Likewise, parasites resistant to trifluralin exhibited a point mutation in nucleotide position 1850 of α -tubulin resulting in a thymine to cytosine substitution. Translation of this mutated sequence revealed a substitution of methionine with threonine at amino acid 268. These mutations are similar to known mutations that confer resistance to oryzalin (another dinitroaniline) in *Toxoplasma* or goosegrass. To confirm that these point mutations are responsible for the drug resistance phenotype, the mutated α -tubulin gene associated with trifluralin resistance was cloned into a *Toxoplasma* expression vector and electroporated into wild-type parasites. Transformed parasites were subjected to drug selection (using trifluralin), and indeed converted from wild-type to the resistant phenotype. Further studies are needed to analyze the mechanism of the insertion of the transgene. Parallel experiments will be performed using the benfluralin-associated mutation. Our studies should help to elucidate the mode of action of these dinitroaniline herbicides in *Toxoplasma* and their utility as inhibitors of the parasite cytoskeleton.

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FRIENDLY IRON AND ADVERSARY ZINC MODULATE TROPHOZOITE GROWTH OF *ENTAMOEBIA HISTOLYTICA*

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Entamoeba histolytica has cytosolic fermentation enzymes possibly acquired through horizontal gene transfer from bacteria to survive within the human intestine. This protist constitutes an ideal model to study the origin and evolution of anaerobic metabolism. *Entamoeba histolytica* alcohol dehydrogenase 2 (EhADH2) is a fusion-protein with N-terminal ALDH and C-terminal ADH domains and is essential in glycolytic fermentation of *E. histolytica*. We have identified a member of the ADHE family from *Entamoeba invadens*, a reptilian and amphibian pathogen. Expression of EhADH2 and EiADHE restored the ability of a mutant *E. coli* strain to grow anaerobically. EiADHE showed ADH and ALDH activities with higher resistance to oxygen inactivation. Molecular and biochemical studies demonstrate these bi-functional enzymes require iron for both ADH and ALDH activities. Zinc and chelators dramatically reduced enzymatic activities and anaerobic growth of the recombinant *E. coli* strains transformed with pEhADH2 and pEiADHE. Addition of iron to *E. coli*_{pEhADH2} and *E. histolytica* trophozoites significantly enhanced bacterial and amoebic growth while zinc or chelators inhibited or halted it. We are currently testing metal effects on *E. invadens* trophozoites. These results confirm homology to bacterial alcohol dehydrogenases such as AdhII from *Zimomonas mobilis*, a "unifunctional" alcohol dehydrogenase. Comparisons between ADHE enzymes (human, reptilian, amphibian pathogens or commensal protists) will provide insights to their evolutionary history and host immune responses.

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MOTILE BEHAVIOR OF *TRICHOMONAS*: LACK OF RESPONSE TO OXYGEN GRADIENTS, AND FRACTAL DISPERSION

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Log-phase *Trichomonas vaginalis* cells form dynamic clusters in the presence of 10% horse serum. Cells under a cover slip form such clusters uniformly throughout the medium, including next to the meniscus at the edge of the cover slip, where oxygen gradients would develop. Among free-swimming cells (those not involved in the dynamic clusters), no taxis or kinesis, or change in concentration was observed at the meniscus. Measurements of the mean square displacement of the free-swimming cells yielded a power law, suggesting that random dispersion of these cells is best described by a fractal Levy walk distribution, rather than the more familiar Gaussian distribution. The potential adaptive significance of such behavior will be considered.

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IDENTIFICATION AND CHARACTERIZATION OF ARF GUANINE NUCLEOTIDE EXCHANGE FACTORS OF THE SEC7 FAMILY IN *TETRAHYMENA THERMOPHILA*

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Sec7 GEFs are known to activate small G proteins of the ARF (ADP ribosylation factors) family, which function to regulate cellular functions such as vesicular trafficking, microtubule dynamics and development. In 1999, a guanine nucleotide exchange factor (GEF) was identified in *Paramecium* from a screen for ciliary proteins and was named PSec7 (Nair et al, FASEB J.) Use of the *Paramecium* PSec7 antibody in *Tetrahymena thermophila* recognized a putative GEF protein, Gef1p, which localizes to the cilia in immunofluorescence, immunoEM and western blot experiments. PCR methods and database analysis were used to identify *Tetrahymena* GEF1. A protein 2053 amino acids in length, containing Sec7 motifs, truncated IQ motifs and PH domains, homologous to similar domains in Psec7, was cloned. Upregulation of the product of this gene was observed via RT-PCR following deciliation and subsequent regrowth of cilia in *Tetrahymena* cells. Since these observations, two other GEFs of the Sec7 family have been identified in the *Tetrahymena* genome database. Constructs are currently being made to tag these proteins with either GFP or HA in order to determine the localization of these GEF proteins in *Tetrahymena*.

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INTERACTIONS AMONG MARINE PROTISTS: IMPORTANCE OF MIXOTROPHY, pH AND TOXINS

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Our knowledge about the interactions among free-living protists in the marine pelagial has increased dramatically during the past 25 years. In the early 1980s the concept of the microbial loop was presented and quickly adopted by aquatic scientists. During the following years the quantitative importance of especially heterotrophic protists as grazers on bacteria and algae became evident. Initially heterotrophic protists were treated in simple size categories. However, it soon became clear that the heterotrophic protists constituted a functionally very diverse group with all kinds of specializations in feeding mechanisms making them fit into different niches. Historically, marine protists were grouped into phototrophs (= algae) and heterotrophs (= protozoa). During the 80s and 90s it was realized that such a clear distinction often was not possible, because many protists combine photosynthesis and food uptake in varying degrees. It also became clear that marine protists interacted with each other chemically. The pH of seawater is often regarded as being fairly constant around pH 8.1. However, during algal blooms, the uptake of inorganic carbon due to algal photosynthesis will elevate pH of the seawater, and it reaches levels of up to pH 9 and 9.5. Some marine protists are quite sensitive to high pH levels and thus such high pH levels will lead to species succession among both phototrophic and heterotrophic protists. Some bloom forming algae produce toxins, which kill competitors and grazers. Although quite a few toxins have been identified and characterized, experiments have shown that none of these known toxins are implicated in the toxic effects observed on other protists. Thus new and yet undescribed toxins are without doubt to be discovered.

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“EXTREMOPHILE” FORAMINIFERA AND H, N, C, AND S CYCLING

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The study of extremophiles lends insight into environmental limits of life today, in the past, and, potentially, on other planets. Thus, research on extremophiles is relevant to evolutionary biology, ecology, biogeochemistry, and astrobiology. Although foraminiferan protists are typically considered to be aerobes, observations from the past two decades suggest that at least some species are facultative anaerobes capable of surviving lengthy periods of anoxia (i.e., lack of dissolved oxygen) and sulfide enrichment (e.g., exceeding 10 μM). This talk will focus on foraminiferal inhabitants of two so-called “extreme” environments: anoxic, sulfidic sediments and sediments of the redox boundary. Additional harsh chemical constituents exist in these habitats, including reactive oxygen species. An overview will be presented of the cell ultrastructure and ecology of these extremophile benthic foraminifers, which occur in sediments of silled basins, gas seeps, and fjords. The observed cell biological strategies that these protists have employed to survive these extreme environments include the presence of endosymbionts or ectosymbionts, sequestration of functional chloroplasts, and proliferation of peroxisomes and endoplasmic reticulum. Commonly, mitochondrial distributions in these foraminifera are unusually structured (e.g., at the cell periphery, in dense groupings within the cell, and/or associated with endoplasmic reticulum). Because multiple biogeochemical reactions occur in the redox boundary, it is an important zone to study in the context of nutrient cycling. For example, denitrification has recently been demonstrated in certain foraminiferal inhabitants of redox boundary sediments. Discussion will be presented on the potential impacts that extremophile foraminifera have on biogeochemical cycling. Additional topics will include a paleontological perspective, in the context of early eukaryotic diversification and synecology.

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METHANE FROM PROTISTS AND METHANE FOR PROTISTS: THEIR CONTRIBUTION TO METHANE PRODUCTION, AND THEIR EFFECT UPON METHANE OXIDATION

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In most freshwater ecosystems, methane is the end product of anaerobic mineralization. In flooded soils and sediments it diffuses from the anoxic deeper parts towards oxic-anoxic boundaries where a significant fraction is oxidized before it can reach e.g. the atmosphere. Methane deserves attention for two reasons: it is an important compound in carbon cycling between anoxic and oxic environments, and it contributes significantly to global warming. Here we will deal with the role of protists in methane cycling. In anoxic environments, methanogenic endosymbionts e.g. of ciliates may contribute to overall methanogenesis, and protists may graze on free-living methanogens potentially influencing number and/or activity. We studied these effects in a flooded rice field soil and found the contribution of methanogenic symbionts to overall methane production being a transient phenomenon, restricted to the first two weeks after flooding. Similarly, the ingestion of methanogens by anaerobic ciliates could be demonstrated, but seems to be a less important factor for methanogenic populations. Again in flooded rice field soils, we studied grazing effects on methane oxidizing bacteria. Methane oxidation is the key process controlling methane emission from anoxic habitats into the atmosphere. A specific group of aerobic bacteria oxidizes methane and is also able to assimilate methane-C. We used microcosms simulating the oxic-anoxic boundary layer to expose a rice field soil to opposing gradients of oxygen and methane. Using ^{13}C -labelled methane and stable isotope probing, ^{13}C -enriched “heavy” RNA could be retrieved. Molecular fingerprints and cloning this “heavy” RNA revealed sequences affiliated to methane oxidizing bacteria, but also to the scavenging prokaryotic Myxobacteria, and to protistan grazers including specific representatives of amoebae, ciliates, and flagellates. Ongoing work is focused on the specificity of prey-predator relations between methanotrophic bacteria and protists. Comparative studies in other environments are in progress.

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CARBONATE PRODUCTION IN THE WORLD'S OCEAN: THE ROLE OF FORAMINIFERA

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Foraminifera are among the most abundant organisms in the world's ocean and their shells represent a major and globally significant sink for calcium carbonate. Because the oceanic carbonate system is intimately linked to both atmospheric CO₂ and the global carbon cycle, carbonate production of foraminifera holds great interest for paleoclimatologists. Despite their abundance, the paucity of foraminiferal carbonate production rate estimates has previously precluded production calculations at the global level. Therefore, the significance of foraminifera in the global ocean carbon cycle has remained subject to speculations. In order to assess the contribution all benthic and planktic foraminifera make to the annual global calcium carbonate budget, we have developed a model that recalculates foraminiferal carbonate production rates from sea surface-, sediment trap-, flux- and benthic/plankton ratio- data. We then calculated global carbonate production for both benthic and planktic foraminifera using areal data and estimated average production rates. Our first order production estimate shows that benthic and planktic foraminifera contribute more than a billion tons of CaCO₃ per year. This represents approximately 24.4 % of the global ocean carbonate production. Therefore, foraminifera play a significant role in the present-day carbonate budget of the world's oceans. Eighty-five percent of the global foraminiferal carbonate production is estimated to be produced by planktic foraminifera alone. However, a large portion of this production may be redissolved fairly quickly after it is produced. Our first order estimate provides a global perspective on the contribution of foraminifera to ocean carbonate production and should help to identify aspects of carbonate production requiring additional examination and research.

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THE ROLE OF PROTOZOA IN OCEAN IRON BIOGEOCHEMISTRY

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Single-celled microzooplankton are significant grazers of phytoplankton in marine environments. Phytoplankton growth rates are often balanced by grazing mortality rates in many pelagic ocean ecosystems over varying spatial and temporal scales. As a result, protozoa can be a significant source of recycled nutrients. Nutrient regeneration is particularly important in oligotrophic environments with relatively small external nutrient inputs. Iron has been shown to limit phytoplankton growth in large regions of the global ocean, with significant biogeochemical implications. Models of iron cycling indicate that recycled sources may be important, however recycling rates have been difficult to measure directly. Furthermore, little is known about the chemical form or bioavailability of iron that is excreted from marine protozoa. Results will be presented from laboratory and field experiments in the Southern Ocean and equatorial Pacific Ocean designed to elucidate the importance of protozoa grazing processes to ocean iron biogeochemistry. Measurements of iron in individual phytoplankton and protozoa cells collected from surface waters have been combined with group-specific grazing rates to calculate remineralization rates. Laboratory culture experiments with model organisms have been conducted to determine the iron-binding characteristics and bioavailability of recycled iron. These results will be discussed within the context of current research into ocean biogeochemistry.

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DINITROGEN FIXATION BY A MARINE PHYTOPLANKTON ECOSYSTEM

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A previously undescribed filamentous non-heterocystous cyanobacterium (*Leptolyngbya nodulosa*) isolated from the South China Sea is unique in its ability, at low light intensities, to form discrete nodular structures (nodules). Axenic cultures could be grown in defined medium only in the presence of a combined nitrogen source, while non-axenic cultures grew indefinitely in media without any source of combined nitrogen. A DNA sequence identified as a portion of *nifH* was amplified from non-axenic cultures, but could not be amplified from axenic cultures. This sequence was similar to *nifH* genes from marine bacteria, and less similar to sequences from dinitrogen-fixing cyanobacteria. Nitrogenase activity, as measured by the reduction of

acetylene to ethylene, demonstrated a diurnal cycle of dinitrogen fixation only in darkness. Activity was most pronounced under anaerobic conditions, but was substantial under 25% of oxygen-saturated air. Concentrations of DCMU that inhibit photosynthesis completely reversed the diurnal cycle, with dinitrogenase activity occurring only during light. When the protein synthesis inhibitor chloramphenicol was added during the light period (no nitrogenase activity), it blocked the expression of nitrogenase during subsequent darkness. However, when chloramphenicol was added in darkness to cultures previously treated with DCMU (no nitrogenase activity), then nitrogenase activity was expressed during the subsequent light period exactly as when no chloramphenicol was present. Fluorescence optical microscopy and transmission electron microscopy indicated bacteria under the thick sheath of *L. nodulosa*. These data collectively indicate that *L. nodulosa* does not fix dinitrogen, but occurs symbiotically with dinitrogen-fixing heterotrophic bacteria. A model is proposed to explain the role of nodules in this relationship.

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STEPS TOWARDS UNRAVELING THE REGULATION OF NITROGEN ASSIMILATION IN THE MARINE DIATOM *THALASSIOSIRA PSEUDONANA*: OSCILLATIONS IN MRNA LEVELS OF FIVE KEY NITROGEN-ASSIMILATING ENZYMES

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Marine diatoms are dominant members of phytoplankton communities in coastal upwelling systems, reflecting their physiological potential to rapidly exploit upwelled nutrients, particularly nitrate. We identified genes encoding five key nitrogen assimilating enzymes in the genome of the marine diatom *Thalassiosira pseudonana* and examined the influence of nitrate and ammonium on the diurnal expression of each gene using quantitative reverse transcriptase PCR (QRT-PCR). As in other photosynthetic eukaryotes, nitrate is reduced to nitrite in the cytosol of diatoms by nitrate reductase (NR, encoded by *nirB*). Following a shift to nitrate or ammonium, a diurnal oscillation in *nirB* abundance was observed, similar to patterns reported for NR abundance and activity. The diurnal oscillation was abolished when cells were transferred to continuous light, indicating that *nirB* is not circadian controlled. In contrast to vascular plants, two nitrite reductase genes were identified in the *T. pseudonana* genome: a chloroplast-targeted ferredoxin-dependent enzyme (*nir1*), homologous to *nir1* of vascular plants, and a cytosolic NAD(P)H-dependent enzyme (*nir2*), homologous to fungal and bacterial nitrite reductases. *Nir1* was detected in both nitrate- and ammonium-grown cells and the diurnal pattern of expression was correlated with *nirB* levels in each culture condition. In contrast, transcript levels of *nir2*, and the chloroplast- and cytosolic-targeted glutamine synthetases (*gln1* and *gln2*, respectively) were similar in nitrate- and ammonium-grown cells during the first 24 h following the transition to fresh medium. In the following 48 h, *nir2*, *gln1*, and *gln2* abundances were generally higher in nitrate- than ammonium-grown cells, however, patterns of diurnal expression were similar in all cultures. Based on patterns of gene expression, we propose nitrate is assimilated into organic molecules in both the cytosol and chloroplast of diatoms and that the enzymes encoded by *nir2* and *gln2* contribute to the ecologically important dark assimilation of nitrate observed in marine diatoms.

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DIDYMOSPHENIA GEMINATA AS A NUISANCE DIATOM: RUNAWAY STALK PRODUCTION RESULTS IN MATS WITH SIGNIFICANT ENVIRONMENTAL IMPACT

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The diatom *Didymosphenia geminata* (Lyngbe) Schmidt is emerging as a significant nuisance organism with an extraordinary capacity to impact stream ecosystems on a global scale. In recent years, streams in New Zealand, North America, Europe, and Asia have been colonized by unprecedented masses of “didymo”. This diatom is able to cover up to 100% of surfaces with thicknesses of greater than 20 cm, greatly altering physical and biological conditions within streams. The impact of *D. geminata* is primarily due to the production of prodigious amounts of extracellular polymers organized into stalks. The capability to secrete large quantities of stalk material differentiates *D. geminata* from other related benthic diatoms. When cells divide, the stalk bifurcates, the end result of which is an overall branched structure with stalks intercalating and coalescing to

form aggregate “woven fabric” mats that trap algae, macroinvertebrates, detritus and other stream debris and which, at a macro scale, resemble raw sewage and are quite resistant to degradation. Stalk macromolecular organization was examined as a first step toward determination of the mechanism whereby stalk production has been “turned on” in recent nuisance blooms worldwide. The stalk is composed primarily of sulfated polysaccharides with significant uronic acid content and protein. Galactose, xylose, and mannose were the major neutral monosaccharides with the predominate glycosyl linkages 3,4-Gal, 4,6-Man, and 4/5-Xyl. Striated parallel bands were highlighted after staining for anionic polysaccharides and sulfated polymers. Sequential extraction yielded significant EDTA soluble material, and, although it did not result in complete dissolution, caused stalks to loosen and unravel. Our results indicate a sulfated xylogalactan as the major component of *D. geminata* stalks, which has chemical similarities to the stalks of benthic diatoms *Cymbella mexicana*, *Cymbella cistula*, and *Gomphonema olivaceum*. Investigation into *D. geminata* carbohydrate metabolism and glycan synthase activity may reveal causes of the worldwide upsurge in stalk production during blooms of *D. geminata* in recent years.

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NITRATE UPTAKE RATE IN *PORPHYRA* SPECIES FROM DIFFERENT TIDE LEVELS: RESPONSES TO DESICCATION STRESS

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We have reported that an intertidal seaweed, *Porphyra umbilicalis*, with desiccation stress had very similar nitrate uptake rate when compared with continually submerged tissues. This suggested that species in the intertidal zone, which have longer exposure times, may have higher time-use efficiency than the subtidal species in terms of nitrate uptake. A correlation between the degree of stimulation of N uptake following emersion and intertidal distribution may exist; upper-shore species may exhibit greater stimulation of N uptake following desiccation, may achieve maximum uptake at higher levels of desiccation, and may be able to take up nitrogen following severe desiccation that inhibits uptake by low-shore species. To test these hypotheses, different *Porphyra* species from different tide levels were tested, *P. umbilicalis* from mid-intertidal zone and *P. leucosticta* from lower intertidal zone. Both species were cultivated in a greenhouse under natural light intensities, at 10 °C and given 30 µM nitrate and 3 µM phosphate. During 5-7 days acclimation, tissues were exposed twice per day using a tide simulating apparatus to generate tissue water losses of 0%, 30-50%, and 85%-95%. Under moderate water loss, nitrate uptake rate by both species was not significantly different with the rate of continually submerged tissue. Nitrate uptake by *P. umbilicalis* fully recovered within four hours after severe desiccation treatment. The uptake rate remained high until dark when the uptake rate of continually submerged tissue dropped. However, the nitrate uptake by *P. leucosticta* was not stimulated by severe desiccation stress. These results suggest that the recovery of nitrate uptake rate after periods of desiccation may also be another key factor in determining the zonation of algae on the shore.

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COMPARATIVE EFFECTS OF HERBICIDES ON PHOTOSYNTHESIS AND GROWTH OF THE TROPICAL MICROALGA *NEPHROSELMIS PYRIFORMIS*

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PSII inhibiting pesticides have been discovered in sediments along the Queensland coast. To predict flow on effects to benthic marine communities, it is important to evaluate effects on phyto-benthic organisms. *Nephroselmis pyriformis* was isolated from the benthos of Rowes Bay, Townsville, Australia (Latitude 19 15.0' S, Longitude 146 50.0' E) and taken into monoclonal culture at NQAIF (NQAIF 117). Cultures were maintained at 24°C in Guillard's (f/2) marine medium at a 12:12 hour light:dark cycle with an irradiance of 43 µmol photons m⁻² s⁻¹ for at least six months prior to experimentation. Dose response experiments were conducted for the PSII inhibiting herbicides diuron, atrazine, and hexazinone for chronic (3-5 days, diuron:

0.1-73nM; atrazine: 14-3,000nM; hexazinone: 2-2,600nM) and acute (6 min; diuron: 0.43-82nM; atrazine: 2.6-750nM; hexazinone-6 min: 1.2-584nM, hexazinone-240min: 0.3-73nM) exposures. Chronic effects of these herbicides were measured using percent growth (OD750nm) and percent PSII yield (Mini-PAM) as endpoints, while acute effects measured percent PSII yield with an imaging PAM (I-PAM). 3-day percent growth EC_{50s} were $25 \pm SE2$, $160 \pm SE12$, and $33 \pm SE1$ nM for diuron, atrazine, and hexazinone, respectively. 3-day percent PSII yield EC_{50s} were $25 \pm SE1$, $130 \pm SE6$, and $24 \pm SE0.5$ nM for diuron, atrazine, and hexazinone, respectively. 6-min percent PSII yield EC_{50s} for acute exposures were, $8.9 \pm SE0.2$, $66 \pm SE2.1$, and $72 \pm SE3.7$ nM for diuron, atrazine, and hexazinone, respectively, while the 240-min percent PSII yield EC_{50} for hexazinone was $9.5 \pm SE0.2$ nM. Acute and chronic toxicity measurements were extremely sensitive for all endpoints and effects were recorded at environmentally relevant concentrations for diuron. Chronic exposure results showed that except for diuron, the 3-day percent PSII yield was more sensitive than percent growth. However, growth of *N. pyriformis* partially recovered after 5 days without exchange of media, while percent PSII yield did not. This may indicate that *N. pyriformis* could rescue growth by switching to heterotrophic nutrient acquisition.

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PHYLOGEOGRAPHY OF *ASPARAGOPSIS TAXIFORMIS* (BONNEMAISONIALES, RHODOPHYTA) IN THE HAWAIIAN ISLANDS: TWO mtDNA MARKERS SUPPORT THREE SEPARATE INTRODUCTIONS

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Fifty samples of the gametophyte stage of *Asparagopsis taxiformis*, principally from the Main and Northwestern Hawaiian Islands, were obtained from field collections and archived herbarium material. Of these, 36 were successfully sequenced for the mitochondrial *cox2-3* spacer region, and 42 for the mitochondrial COI gene. Statistical parsimony analyses of both regions for the Hawaiian collections revealed relationships that were consistent with those previously published for global *A. taxiformis* samples, which also included a number from the Hawaiian Islands. The Main Hawaiian Islands were dominated by lineage 1, which is known from the Pacific coast of Central America and the main Hawaiian Islands. The Northwestern Hawaiian Islands, with one exception, contained a single widespread lineage (2) that is known from the Indo-Pacific, central Mediterranean and southern Portugal. A third lineage (4) was present in Hawaii only along a localized region of the south shore of Oahu, but was widespread in tropical and subtropical waters outside of the Hawaiian Islands. Based on collection dates from archived herbarium material and the distributional trends of the lineages within the Hawaiian Islands, lineage 2 is hypothesized to be the original "native" lineage of this species in the Hawaiian archipelago, and lineage 4 may have arrived as recently as 2006. COI and *cox2-3* spacer sequences were always in accordance for lineage assignment of *A. taxiformis* samples, and future studies may only need to sequence one of the two markers.

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SUBMERSED PLANTS OF THE INDIAN RIVER LAGOON: A FLORISTIC INVENTORY AND FIELD GUIDE

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The Indian River Lagoon system (IRL), the longest barrier-island/tidal-inlet system in the continental United States, spans more than one-third of Florida's east coast. The IRL has often been called the most biologically diverse estuary in North America, which is believed due to a favorable climate and contributions from both warm temperate and tropical biotas. While the importance of seagrasses (7 species) in the IRL is widely recognized, there has been remarkably little research dealing with the marine algae of the central east coast of Florida, including the IRL; previously, macroalgae have largely been overlooked in the IRL, with existing checklists based on limited studies, published primarily in the 1960s and 1970s. Given the rapidly changing environments of the IRL, it is essential that a biodiversity inventory and baseline be established for submerged plants. To address this void, our goal was to produce a comprehensive floristic field guide for use by researchers, educators, resource managers, and the general public. Our intensive sampling of habitats throughout the IRL has resulted in a field guide of nearly 250 species of submerged plants. The description, along with distribution, taxonomic, and other information, and multiple underwater color macro-images, are

presented for each plant on a single page, often richly illustrated with photomicrographs and line drawings depicting the habit and anatomical features. The IRL flora is dominated by the Rhodophyceae (117 species), the Chlorophyta (64 species), and Phaeophyceae (39 species). The field guide identifies a number of range extensions of algae into the IRL and new records for Florida and the Western Atlantic. Publication of the field guide will occur in early 2008. Besides substantially adding to what is currently known about these ecologically important organisms, the field guide should stimulate additional research and interest on submerged marine plants by resource managers, conservationists, and the broad scientific community, and serve as educational/recreational tools for the interested public.

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EUROPEAN INFLUENCES ON THE NORTH AMERICAN SHORE

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The common European rockweed *Fucus serratus* was introduced into North America sometime before 1869, when it was found by Fowler at Pictou, Nova Scotia. Its colonization of Nova Scotia, Prince Edward Island, and New Brunswick was last reported in the early 1970s and will be updated here. Among other expansions and secondary introductions, only a small part of the southeastern shore of Cape Breton now lacks *F. serratus*. Several European locations have been suggested as the place of origin for the introduction, which may have occurred via rock ballast. To examine this, we compiled historical records of European shipping traffic into Pictou from 1773-1861 (e.g., customs' records, weekly arrivals of ships reported in the Shipping Intelligence of *The Nova Scotian*, *The Colonial Patriot*, and *The Eastern Chronicle*), collected *F. serratus* from locations at or near ports in Scotland and Ireland that the shipping analysis suggested to be important, and genotyped individuals from these places and three sites in Nova Scotia (including Pictou) at 7 microsatellite loci. These genotypes were incorporated into a large existing data set for European *F. serratus*, and assignment tests were used to determine source locations for Nova Scotian *F. serratus*. *Fucus serratus* from Pictou was assigned to Galway, Ireland, and *F. serratus* from two populations in Cape Breton (Inverness and Caplin Cove, N.S.) was assigned to Greenock, Scotland. Thus, at least two introductions of *F. serratus* into Nova Scotia occurred from the British Isles, and it is likely that other species were co-introduced. In particular, we are using several molecular markers to examine whether some portion of the North American population of the snail *Littorina littorea* was co-introduced with *F. serratus*; *L. littorea* was first observed in abundance in the Gulf of St. Lawrence in the mid-1800s and spread to Cape May by the late 1800s. (Supported by grants from the National Science Foundation and National Geographic Society to S.H.B. and L.E.J. and by a NSF Research Coordination Network grant, CORONA, to C.C.).

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THE PROGRESSION OF THE INVASIVE RED ALGA *GRATELOUPIA TURUTURU* YAMADA ALONG THE CONNECTICUT COASTLINE IN LONG ISLAND SOUND, USA

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The introduced red alga *Grateloupia turuturu* Yamada was first found in Narragansett Bay, Rhode Island, USA in 1994. In 2004, it was discovered in Waterford, Connecticut, and in 2006 in Groton, CT. *Grateloupia turuturu* prefers rocky, low intertidal and shallow subtidal habitats, and co-occurs with the native red alga, *Chondrus crispus* Stackhouse. Monthly monitoring of *G. turuturu* and *C. crispus* along a subtidal cobble beach and adjacent rocky intertidal platforms at Waterford will provide a basis for comparison of the population dynamics between these ecologically different habitats. Quadrat sampling has been used to estimate the cover of the different macroalgal species over the past year on the cobble beach, and recently on the adjacent rocky platforms. Since the Waterford population is located near the seawater outflow of the Millstone Nuclear Power Plant, site temperature is approximately 2°C above surrounding Long Island Sound temperature. We are also

examining the reproductive phenology of *G. turuturu*, and tracking spore output and dispersal from the Waterford population as indicators of the potential for spread by *G. turuturu* throughout Long Island Sound.

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VARIATION IN WAVE FORCES ALONG VERTICAL TRANSECTS IN THE WAVE-SWEPT ROCKY INTERTIDAL ZONE AS A POTENTIAL DRIVER FOR MACROALGAL DISTRIBUTION

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Macroalgae living in the wave-swept rocky intertidal zone must contend with both biological and physical stresses. Among the physical stresses, desiccation and temperature extremes during low tides and hydrodynamic forces from breaking waves are thought to have the greatest influence on the distribution and abundance of species. Although much attention has been paid to the vertical gradients in temperature and desiccation, vertical patterns of wave force in the intertidal zone have not been thoroughly investigated. This leaves open many questions regarding the influence of wave forces on macroalgal dislodgement, fatigue of tissues, gamete and propagule dispersal, productivity, and herbivory intensity. To explore vertical patterns of wave force, we designed and installed custom, autonomous digital wave-force dataloggers along vertical transects with different shoreline slopes at Hopkins Marine Station. Over 100,000 individual waves were measured across a variety of sea states and tidal cycles. Opposite patterns of force were observed between the two transects where, on average, wave force was positively correlated with height on the steep slope, but negatively correlated with height on the gentle slope. These results suggest topographic variation, e.g. slope, can strongly influence the patterns of hydrodynamic force among nearby locations and even within what might be considered a single site. The ecological consequences of these patterns on macroalgal abundance and herbivory will be discussed.

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PHYLOGENY, SYSTEMATICS AND BIOGEOGRAPHY OF THE RED ALGAE

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Phylogenetic and morphological evidence support the opinion that families and tribes of higher red algae (Class Florideophyceae, Subclass Rhodophycidae) originated in the Southern Hemisphere in Gondwana during the Mesozoic and early Tertiary with relict species found primarily in the vicinity of Australia and New Zealand. Groups that originated on the western side of Australia prior to the marine transgression across the Tasmanian land bridge that connected Australia and Antarctica spread through the Tethyan and Indo West-Pacific Oceans into the Atlantic and as far west as the coasts of Pacific North and South America, or migrated along the edge of the Western Pacific to Japan and East Asia. Those that originated in the southern Pacific Ocean on the eastern side of Australia or in New Zealand were distributed westward along Antarctica to South America and north to Pacific North America reaching Alaska and East Asia, or migrated through West Antarctica to southern Africa with some species undergoing amphitropical distribution to Western Europe. Species that reached the North Pacific underwent further radiation in the vicinity of the Bering Sea and the Sea of Okhotsk where some of these underwent trans-Arctic distribution from the North Pacific to the North Atlantic Ocean, primarily after the opening of the Bering Strait. None of the taxa that reached Europe or Atlantic North America from southern Africa or that originated within the Tethyan Ocean underwent trans-Arctic distribution at a later date. The importance of vicariance events due to plate tectonics and sea floor spreading and paleoclimatic changes to the historical biogeography of Rhodophycidae will be discussed in comparison to that of long-range dispersal by currents.

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EVOLUTIONARY BIOGEOGRAPHY OF SIPHONOUS GREEN ALGAE

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The Bryopsidales are a group of green macroalgae with thalli consisting of a single, giant, tubular cell. These tubular cells (= siphons) branch and anastomose in various ways, yielding a variety of thallus forms ranging from very simple to highly complex. This makes the Bryopsidales a suitable target for studying the evolution

of algal thallus complexity. The evolutionary history of the bryopsidalean algae has been studied with molecular phylogenetic tools. We will present the latest insights into bryopsidalean diversification based on multi-marker phylogenetic analyses. We will define the major lineages of the group, address issues concerning genus non-monophyly and discuss the evolution of thallus complexity. The bryopsidalean genera *Codium* and *Halimeda* have a broad distribution and constitute ideal model systems for studying speciation history and historical biogeography. We will present molecular phylogenetic hypotheses of these genera and interpret their evolutionary diversification from geographic and macroecological perspectives, using novel techniques involving phylogeographic analyses of georeferenced collections and satellite image analysis in a GIS framework.

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OUR CURRENT UNDERSTANDING OF BROWN ALGAL EVOLUTION

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Studies using DNA sequence data in the last decade have drastically changed our view of brown algal evolution. Morphologically simple ectocarpaleans were hypothesized to make an early divergence but instead turned out to be a recent group and sister to the kelps. To make orders monophyletic some taxa needed to be moved to another order or assigned to a new order. Most orders have been delineated by now. However, only a few sister-order relationships have been detected until today. The recently reinstated order Discosporangiales with two monotypic families is the most basal brown algal order, probably followed by the recently created Ishigeales. However, whether the other basal orders Dictyotales, Syringodermatales and Sphacelariales form a monophyletic clade or a grade is still unclear. All other orders belong to a clade often referred to as the brown crown radiation. Resolving the ordinal relationships within the base and within the crown of the brown algal tree remains the current challenge and a multiple gene analysis combined with a comparison of the gene order in chloroplast and mitochondrion will be the approach. Currently most studies are on a lower taxonomic level. Special attention will be given to phylogenetic explorations in the fuclean genus *Cystoseira* and related genera in the family Sargassaceae. This worldwide temperate to tropical genus with its centre of diversity in the Mediterranean actually consists of six separate clades, i.e., three European and three Indo-Pacific clades and each merits genus-level status considering the amount of DNA sequence variation compared to other sargassacean genera. Mediterranean *Cystoseira* is taxonomically difficult and is considered undergoing active speciation. This may mean that the phylogeny can never be completely resolved and also the limit of DNA barcoding is reached here.

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THE INTERNATIONAL CENSUS OF MARINE MICROBES AND MARINE PROTISTS

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The International Census of Marine Microbes (ICoMM) is one of 14 ocean realm projects of the Census of Marine Life Program (CoML) that seeks to assess and explain the diversity, distribution, and abundance of marine microbial life (Bacteria, Archaea, Eukarya and their associated viruses) in the oceans -- past, present, and future. ICoMM is a joint venture between The Royal Netherlands Institute for Sea Research (NIOZ) and the Marine Biological Laboratory (MBL) in Woods Hole, Massachusetts. The ICoMM Secretariat at Woods Hole hosts the website <http://icomm.mbl.edu> and the distributed database network MICROBIS, designed to bring together molecular, environmental, geospatial, and taxonomic information within a framework that integrates into OBIS - the database that serves the entire CoML program. Since 2005, ICoMM has sponsored meetings for four primary working groups (Benthic, Open Ocean and Coastal Systems, Technology, and Informatics and Data Management) and its Scientific Advisory Council. ICoMM has recently formed a new working group to address the specific challenges associated with census issues surrounding marine protists. Protists provide a unique opportunity to study biogeography and biodiversity of marine microbes because of a wealth of legacy data (both molecular and morphological in nature) collected over decades of research, combined with more recent environmental molecular (i.e. SSU rRNA gene) surveys. The challenge of compiling and centralizing these data along with applying enabling technologies that will allow for large-scale comparative global protistan diversity studies in marine environments will be discussed including progress on developing SSU RNA gene "tag" sequencing methods targeted at eukaryotes. Although a complete census is

most likely beyond our grasp, the scientific return will be considerable if the information is integrated with contextual information that can inform us about the interplay between microbial eukaryotes and their environment.

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THE BARCODE OF LIFE INITIATIVE

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DNA barcoding aims at using short standardized portions of the genome for fast and accurate identification of specimens at species level and to facilitate the discovery of unknown species. As such, DNA barcoding can powerfully contribute to taxonomic and biodiversity research. Although the barcode of life initiative is relatively new, several studies have established the utility of a 650 base fragment of the 5' region of mitochondrial gene cytochrome c oxidase I (COI) as a DNA barcode in large taxonomic assemblages--mainly in the animal kingdom—with over 95% resolution at species level. And large-scale barcoding campaigns such as global projects for birds and fishes are underway. As for the protists, COI barcodes have successfully been used in exemplar groups such as macroalgae and other groups are under investigation. The construction of a global on line barcode data management and analysis system and the establishment of high throughout analytical protocols have further facilitated the progress of the barcode of life initiative.

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CAPTURING BIOLOGICAL DATA AND EXPERTISE WITH XML

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An often-overlooked aspect of the so-called “taxonomy crisis” is the orphaning of valuable datasets. Years or decades' worth of information about the distribution and identification of organisms may exist as data trapped in old proprietary databases or unindexed spreadsheets. Posting the data to the Web as a spreadsheet is a partial solution, but spreadsheets are not inherently searchable, and information from one source cannot be easily associated with relevant data from another. XML (eXtensible Markup Language) is a markup system used to describe the structure and semantics of data. XML and its associated specifications allow the user to annotate raw data so that the meaning and context is readily identified. These metadata allow the information to be structured and used in ways that are impossible for noncontextualized data. A well-designed schema can also capture the expertise of the biologist who created the dataset. XML schemas have already been proposed for taxonomy, ecology, RNA structure, and many other biologically relevant areas. XML data is also easily passed into modern databases, and the markup is readable by humans, so it is an effective “rescue” mechanism. However, the process of applying this language to an existing dataset is not trivial, and the design of the data structure is important for success. In this presentation, XML approaches to species curation, taxonomy, and biogeography will be discussed in the context of a real-world dataset (a global survey and meta-analysis of foraminiferal distribution, prepared by a senior foraminiferal taxonomist.)

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PROTISTAN CULTURE COLLECTIONS: THEIR CURRENT STATUS AND THEIR PARTICIPATION IN BARCODING INITIATIVES

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Protists have been grown in culture for approximately 130 years, and public culture collections have existed for many decades. The World Federation of Culture Collections maintains the World Data Center for Microorganisms, and they currently list 522 culture collections in 66 countries. Many of these collections hold primarily bacterial or fungal strains, but there are approximately 200 culture collections that maintain protists. Culture collections typically arise through the efforts of individual scientists, become transformed into public collections and then rise/fall in stature depending upon fluctuating governmental support. The barcode of life is an effort to use a common gene fragment (e.g., COI) that can be used to identify species. Virtually all culture collections are currently supporting barcode initiatives because it holds promise for a rapid and

accurate means of identification and quality control. The ICBN and ICZN use the type method, which anchors a taxonomic name to a specific entity. Ink drawings, EM micrographs or acid-cleaned frustules are designated as types for most microscopic protists, and it is impossible to obtain DNA from these. Therefore, one potential problem for barcoding microorganisms is that of tying the species barcode from a culture strain to a name based on nonDNA yielding type material. The Alfred P. Sloan Foundation recently supported a workshop to address this issue, and the recommendations of the Workshop will be discussed. A 1000 strain challenge by the Canadian Barcode of Life Network arose from the same Workshop. Culture collections around the world are participating in the challenge, which intends to evaluate the breadth and depth of barcodes amongst protists, particularly with regard to the mitochondrial gene CO1. To evaluate breadth, at least one representative from each major protistan group (with mitochondria) is being studied. To evaluate depth, 5 or more strains of a single species are being studied to determine if they have the same or nearly same sequence. Progress on the 1000 strain challenge will be reported.

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MARINE MACROALGAE AS AN EXEMPLAR OF DNA BARCODING AMONG PROTISTAN LINEAGES CHALLENGES WIDELY HELD PERSPECTIVES IN BIODIVERSITY AND BIOGEOGRAPHY

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This presentation will highlight successes to date in our DNA barcoding effort to generate a complete floristic account of the marine macroalgae in Canada. With ca. 2400 barcodes completed to date, we have uncovered 62 overlooked (cryptic) species or records in the Canadian flora including six unique to the low Arctic. In addition to radically altering our perspectives on raw biodiversity (the actual numbers of species in Canadian waters), we are uncovering a bewildering array of tales that are: altering our perspectives on algal distribution at both ecological and geographical scales; challenging widely accepted morphological species concepts; rewriting phylogeographical hypotheses; resolving the dynamic nature of speciation and various hybrid populations; and uncovering key examples of niche exclusion with a corresponding restriction in phenotypic plasticity. I will present an overview of some of the highlights of our work with the aim of conveying the enthusiasm that has gripped our lab in what is truly the golden age of molecular-assisted alpha taxonomy.

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COMPARATIVE PROTISTAN BIOGEOGRAPHY

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The topic of protistan biogeography has generated a great deal of debate in recent years. Intimately tied to this topic is the debate regarding the overall diversity of protists. One side in this argument claims a predominantly global distribution of a relatively few number of protistan species while the opposing side believes that species diversity is vast and that endemism of these species is the rule rather than the exception. In this talk, I suggest that the following two issues fuel this debate. First, a considerable component of this controversy is perceived disagreement rather than real, and stems from the fact that protistologists apply a variety of species concepts when defining species. The inability to employ a single species concept for single-celled microbial eukaryotes complicates analyses of diversity and endemism. Second, regardless of the 'true' diversity, natural protistan assemblages encompass a tremendous breadth of taxonomic/phylogenetic entities. Extant approaches and methods for studying the biodiversity of these assemblages is woefully inadequate, resulting in limited or piecemeal investigations of species diversity and distributions. There is no easy resolution to the issue of protistan biogeography, but a better recognition of the problems involved in research on this topic should improve our understanding of protistan diversity and distribution, and our interpretation of studies on this subject.

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THE EUKARYOTIC TREE OF LIFE ASSESSED THROUGH INCREASED TAXON AND GENE SAMPLING

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A major remaining gap in our knowledge of the tree of life is the uncertain relationship among divergent microbial eukaryotes. Yet knowledge of the phylogenetic positions of microbial eukaryotes is key to understanding the origins of eukaryotes, and where the ancestries of plants, animals and fungi lay within these microbial groups. Eukaryotes are currently classified into six major 'supergroups', the 'Amoebozoa', 'Chromalveolata', 'Excavata', 'Opisthokonta', 'Plantae', and 'Rhizaria'. However, most of the supergroups remain poorly supported by molecular and morphological data. We are increasing taxonomic sampling of multiple genes to assess the broad scaffold of the eukaryotic tree of life and to place enigmatic taxa on the tree. While many well-supported clades are emerging under these analyses, deep nodes remain elusive. At the same time, several 'orphan' lineages - genera with no clear sister genera - have emerged. Hence, we must proceed carefully in proposing hypotheses for eukaryotic taxonomy.

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TESTING THE EFFECTIVENESS OF COX-1 BARCODING AS A TAXONOMIC TOOL TO IDENTIFY *TETRAHYMENA* SPECIES AND TO ELUCIDATE THEIR EVOLUTIONARY HISTORY

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Although it is estimated that there could be as many as 100 million different species on Earth, only 1.5-1.8 million of these have been discovered and described. For this reason, the study of taxonomy should be considered a significant field of research. In terms of protists, many different approaches have been used to elucidate the relationships between different species. Although DNA barcoding using the cytochrome *c* oxidase subunit I (cox-1) gene of the mitochondria has been successfully used in the past to differentiate between animal species, the overall feasibility of cox-1 barcoding of protist species requires more research. The major objective of our project is to determine whether cox-1 barcoding can be used as an effective tool for species identification in *Tetrahymena* by confirming that intraspecific sequence divergence is typically less than 1%. We also aim to use cox-1 sequences to provide the foundation for a population genetics analysis to determine whether any geographic isolation exists in *Tetrahymena* species, or if everything is indeed everywhere as postulated by Finlay and Fenchel. Additionally, cox-1 sequences will be used in conjunction with nuclear SSUrRNA sequences to further clarify phylogenetic relationships and to calculate the coalescent point for *Tetrahymena* species. Initial results suggest that intraspecific sequence divergence is indeed less than 1% in both *Ichthyophthirius multifiliis* (0.6%) and *Tetrahymena hegewischi* (0.8%). Additionally, cox-1 barcoding was used to identify a group of unknown tetrahymenine species, suggesting that this technique can be used to both identify unknown species and to verify earlier taxonomic classifications.

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BARCODING CILIATES - A PHYLUM WITH HIGHLY DIVERGENT COI SEQUENCES

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The mitochondrial cytochrome oxidase subunit I (COI) gene in ciliates is unusual in that it possesses a 108 amino acid long insert, which is not present in other organisms. This insert lies within the barcoding region and shows extreme divergence in its amino acid sequence. Using combinations of several primer pairs, we were able to barcode over 30 species of the Class Oligohymenophorea, representing four subclasses. A comparison of the amino acid sequences shows that the insert is highly variable between species and shows extreme divergence between the different subclasses. The nucleotide sequences are naturally more divergent, as we find numerous silent mutations in the COI gene. Genetic distances among the different ciliate species vary from 0.1 (10% divergence, closely related species) up to 0.6 (60% divergence, species in different subclasses). These data suggest that there is an extreme divergence within the Phylum Ciliophora, as we have so far only analyzed one of the 11 classes. Preliminary results show that the COI gene enables us to detect

cryptic species, which are identical in morphology and possess no or only minimal divergence in their SSrRNA gene and/or the ITS regions.

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'SUPER' PLANT KINGDOM REINSTATED: NONMONOPHYLY OF PRIMARY PHOTOSYNTHETIC EUKARYOTES AS DEDUCED FROM SLOWLY EVOLVING NUCLEAR GENES

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The biodiversity of photosynthetic eukaryotes, traditionally recognized as nine algal divisions or phyla, is attributed to two kinds of endosymbiotic events involving plastids: primary endosymbiosis and secondary endosymbiosis. Therefore, the phylogenetic positions of primary photosynthetic eukaryotes are fundamental for understanding the evolution of eukaryotic cells and establishing higher taxonomic concepts of eukaryotes. Recently, Rodriguez-Ezpeleta et al. (2005, Curr. Biol.) demonstrated the strong monophyly of the three groups of primary phototrophs (green plants, glaucophytes, and red algae) based on 143 nuclear genes (30,113 aa). However, they analyzed only two divisions of the secondary phototrophs belonging to the Stramenopiles-Alveolata (SA) lineage, and their 143 genes included rapidly evolving genes. Since multigene analyses are expected to be increasingly sensitive to long branch attraction, improved taxon sampling and the selection of positions or genes that evolve more slowly have been suggested for resolving deep branching in phylogenies (Philippe & Laurent 1998, Curr. Opin. Genet. Dev.). Here we reexamined the phylogeny of the primary phototrophs based on only 19 slowly evolving nuclear genes (18 genes from the 143 genes, plus hsp90; 5,216 aa) using additional OTUs of Haptophyta and Excavata (Heterolobosea and *Reclinomonas*). The p-distances for each of the 19 genes do not generally exceed 0.4 (based on saturation curves of the distance correction methods [Philippe and Laurent 1998]). Since α - and β -tubulin sequences might be relaxed in eukaryotes lacking flagella (e.g. red algae), and EF-2 sequences might contain unusual phylogenetic information (Stiller et al. 2001, JME), we did not use these three genes. Our phylogenetic results demonstrate the robust non-monophyly of the primary phototrophs and the basal position of red algae within the bikonts, suggesting the loss of plastids in certain eukaryotic lineages under the assumption of the single plastid primary endosymbiosis. Thus the 'super' Plantae (Plant Kingdom) suggested earlier (Nozaki et al. 2003, JME; Nozaki et al. 2003, PSA/SOP meeting) may be reinstated.

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THE RED ALGAL ORDER NEMASTOMATALES: NEW INSIGHTS IN THE LIFE HISTORY, MORPHOLOGY, PHYLOGENY AND BIOGEOGRAPHY OF PERTINENT TAXA

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The order Nemastomatales comprises two families of predominantly gelatinous representatives with heteromorphic life histories, the Nemastomataceae and Schizymeniaceae. New insights in the life history and morphology of pertinent taxa are illustrated with *Platoma cyclocolpum* (Mont.) Schmitz from the Azores, a species in which three modes of thallus development were observed from germinating carpospores. Comparative *rbcL* sequence analysis indicates that species reported as having a wide distribution instead have a restricted distribution. For example, *P. cyclocolpum* may be confined to the Macaronesian islands, and the taxon going under this name in the Indian Ocean (Madagascar) is instead *Platoma chrysymenioides* Gavio et al., a species found throughout the Gulf of Mexico. *Schizymenia dubyi* (Chauvin ex Duby) J. Ag. described from Atlantic France is also present in Japan, but records of this species from the Azores should be referred to *S. apoda* (J. Ag.) J. Ag. described from the Cape Province, S. Africa, and also present in Namibia and Japan. Additionally, new records of recent deepwater collections of *Predaea* and *Titanophora* throughout the Gulf of Mexico, as well as unreported Nemastomatales from the Azores and Japan, are greatly increasing the species diversity of the Order.

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COMPARISON OF THREE ORGANELLE MARKERS FOR PHYLOGEOGRAPHIC INFERENCE IN *BATRACHOSPERMUM HELMINTHOSUM* (BATRACHOSPERMALES, RHODOPHYTA) FROM NORTH AMERICA

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Phylogeographic signal provided by the newly developed 23S plastid rRNA marker (UPA) and the cytochrome oxidase subunit 1 marker (COI) in the freshwater red alga *Batrachospermum helminthosum*, throughout its range in North America, was investigated for intraspecific variation. These markers were compared in individuals from a previous study using the cytochrome oxidase 2-3 spacer region (*cox2-3*), which has yielded the most useful data to date with thirteen haplotypes among geographic locations. Five haplotypes were resolved for the UPA, differing by only 1-2 base pairs (bp), and we conclude that this marker may be more appropriate for studying interspecific variation. In contrast, the COI gene revealed 14 haplotypes, differing from 1-46 base pairs or up to 6.9% sequence variation. The intraspecific variation of COI in this taxon is much greater than that reported thus far for marine red algae (generally <5 bp). The intraspecific variation within *B. helminthosum* is in accord with levels shown in *B. macrosporum* (48 bp within distant locations in Brazil). The COI gene is comparable to the *cox2-3* spacer for phylogeographic studies as the haplotype networks were similar and showed the same geographic patterns. To our knowledge, this is the first comparison of these 3 regions for phylogeographic research in the red algae.

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PHYLOGEOGRAPHIC RELATIONSHIPS AMONG *BATRACHOSPERMUM ARCUATUM* (RHODOPHYTA) COLLECTIONS THROUGHOUT ITS DISTRIBUTION

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The freshwater red alga, *Batrachospermum arcuatum*, has been reported from locations in Europe, Asia, North America and Australasia. Even though it would appear to be cosmopolitan, it has a patchy distribution. For example, in North America, a comprehensive study of historical herbarium sheets and more modern collections showed this species to be restricted to more western locations, particularly the Pacific Northwest. The present research focuses on the relationships among individuals from stream segments in New Zealand, Taiwan, Bulgaria and USA (Hawaii and Washington) using the intraspecific molecular marker, cytochrome oxidase 2-3 spacer region (*cox2-3*). All sequence data were in the range of 375 to 400 bp in length with small indels, but still readily aligned. There were few haplotypes (1-2) per stream segment similar to other *Batrachospermum* species. The relationship of the haplotypes closely mirrored geographic proximity with the haplotypes from Hawaii being most closely related to those from Taiwan and those from North America being more distant. The haplotypes from Bulgaria appeared to be most distant from all others. The lack of shared haplotypes among locations in the collections from around the Pacific may be indicative of either a formerly widespread taxon with relict populations or dispersal with subsequent in situ diversification. Both hypotheses are plausible given the present data.

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THE DINOFLAGELLATE GENOME: INSIGHTS FROM FULL-LENGTH cDNAs FOR *KARLODINIUM* AND *AMPHIDINIUM*

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The gene architecture and function of genomes are poorly understood for the ecologically and evolutionarily

important algae dinoflagellates. Given that their genomes are too large to sequence currently, analysis of expressed genes is the only way to gain information on these aspects. Based on the recent discovery of widespread trans-splicing using a common splice-leader gene, we undertook a project to sequence the full-length cDNAs for *Karolodinium veneficum* (CCMP 2778; 60k clones) and *Amphidinium carterae* (CCMP 1314; 30k clones) to investigate gene content, regulatory elements, and regulatory pathways for cell division, toxin production, and grazing. Conditions necessary for production of billion cell cultures, optimization of cDNA library construction, and preliminary sequencing results will be presented. Initial insights into features of the dinoflagellate genome will be discussed.

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FROM STOP TO START: COMPARING EXPRESSED AND GENOMIC VERSIONS OF GENES IN THE DINOFLAGELLATE *AMPHIDIINIUM CARTERAE*

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Dinoflagellates can have large genome sizes, lack nucleosomes and have acquired plastids from many different lineages. EST projects have shown abundant synonymous substitutions in highly expressed genes suggesting high copy number in the genome. In this project expressed sequences and their genomic counterparts were compared from the peridinin containing dinoflagellate *Amphidinium carterae*. Initially two genes were selected, actin and a keto-yl-reductase (KR) domain gene. The genomic sequences for these genes were amplified and introns were mapped. The actin gene was present in at least two large genomic families with 45 synonymous substitutions between the families but only a single version of the KR gene was found. One intron was found in the actin gene, while the KR gene had 18 introns. Cloning and sequencing of actin gene copies from single cells indicated multiple copies were present in a single cell. Amplification of actin from cDNA indicated biased transcription largely from a single family. By amplifying cDNA using a spliced leader primer and a gene specific primer the splicing of the actin gene was confirmed, although this approach was unsuccessful with the KR gene. Using outwardly directed primers for PCR indicated that actin is found in tandem gene arrays, whereas the KR gene is not. Inverse PCR was used to amplify upstream of the KR gene for 1800 bases without any sign of another gene copy. These differences in genomic context, introns, arrangement and copy number suggest that highly expressed and less highly expressed genes may be differently regulated in dinoflagellates. This same approach of comparing genomic and cDNA versions of genes for diversity and introns, tandem gene arrangement, and the presence of a spliced leader was then applied to a further 45 genes from highly expressed, less highly expressed and singleton categories. Taken together the results suggest that tandem gene arrangement was easily proved for the most highly expressed genes, that intron density is negatively correlated with expression level, and that trans splicing may not be present on less highly expressed genes.

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SEARCH FOR GROWTH-RELATED GENES IN *ALEXANDRIUM FUNDYENSE* THROUGH cDNA MICROARRAY ANALYSIS

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Alexandrium fundyense is a toxic dinoflagellate that causes paralytic shellfish poisoning (PSP). The ecology and oceanography of *A. fundyense* blooms are well-studied, however *in-situ* cell division rate (CDR) which is an important growth parameter in modeling population dynamics remains largely unknown for this species due to technical challenges. One potential approach to resolve the issue is to use cell cycle molecular marker. Correlation between expression of a marker gene and CDR can be established and *in-situ* CDR can then be estimated from measured gene expression data. This research will screen the *A. fundyense* genome for genes associated with the cell division cycle using cDNA microarray. A full-length cDNA library was created using the 22-bp spliced leader RNA specific to dinoflagellates, and from this 1500 random inserts were used to construct a microarray. Abundance of the transcripts at the diel cycle will be compared and candidate genes showing differential expression throughout the cell cycle will be validated using real-time quantitative polymerase chain reaction (RT-qPCR). Optimization of the protocol and results obtained to date will be presented, and the potential applications of the microarray for other studies will also be discussed.

TOWARDS AN IMPROVED UNDERSTANDING OF *CYANIDIALES* ECOLOGY AND THEIR CONTRIBUTION TO BIOGEOCHEMICAL CYCLING IN GEOTHERMAL ENVIRONMENTS

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The thermo-acidophilic, unicellular, and asexual red algal genera, *Cyanidium*, *Galdieria* and *Cyanidioschyzon* belong to the order *Cyanidiales*. They occur almost exclusively in geothermal environments, and are the only photoautotrophs occupying environments with temperatures of 45° to 57°C and pH levels below ~ 4.0. Their sheer numbers, biomass, and brilliant blue-green color establish these eukaryotic algae as a dominant component of microbial communities inhabiting acidic geothermal springs and are especially conspicuous in Yellowstone National Park. Our research in Yellowstone focuses on studying the population diversity and dynamics of the *Cyanidiales* throughout the park. In addition, we have been exploring the biochemistry of these algae, with the aim being to determine if, and to what extent, they may be contributing to the biogeochemical profiles that we encounter in these extreme environments. In one geothermal spring under intense study, we are examining population diversity and dynamics using PCR cloned 18S rDNA, *rbcL*, and microsatellite sequence variation. We have encountered numerous 18S rRNA gene sequences that appear to correspond to several populations of *Cyanidioschyzon merolae*, with the relative dominance of specific populations corresponding to peak ultraviolet irradiance that occurs during the summer solstice. The apparent population dynamics also correspond to the algal mats exhibiting a massive decline, where algal numbers decrease by at least 100-fold, and suggest that UV radiation is a keystone environmental factor that governs the general health and activity of these algae. Work with pure cultures isolated from this spring indicate that these algae are capable of oxidizing arsenite to arsenate and antimonite to antimonate. Further, they are also capable of methylating arsenate to trimethylarsine oxide, and thus likely contribute to the significant amounts of methylated arsenic commonly found in Yellowstone's acidic geothermal environments.

PREY-INDUCED CHANGES IN SWIMMING BEHAVIOR OF PREDATORY DINOFLAGELLATES

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High speed, cinematic, digital holographic microscopy enables tracking of thousands of organisms per mm³ in space and time over a volume with depth extending to more than 1000 times the organism size without loss of resolution. In the current work, we utilize this technique to study the group behavior of mixotrophic and heterotrophic predatory dinoflagellates - *Karlodinium veneficum* and *Pfiesteria piscicida*, respectively, in response to introduction of prey. Data are obtained by simultaneous three-dimensional tracking of thousands of organisms located in a dense suspension with substantial depth of 3 mm. Swimming behaviors are quantified by statistics of radius and pitch of their helical trajectories as well as their translational and angular velocities. The spatial structure of the suspension, such as characteristic distance between predator and prey and among organisms of same species, is determined based on their Nearest Neighbor Distances (NND). The 3-D tracks demonstrate that both organisms perform complex swimming maneuvers. The slower *K. veneficum* moves in both left and right hand helices at velocity in the 20-80 $\mu\text{m s}^{-1}$ range. The faster *P. piscicida* swims only in right hand helices at velocities in the 40-220 $\mu\text{m s}^{-1}$ range. In both cases, Significant changes in behavior and interactions occur as prey is introduced, but trends differ. The slow *K. veneficum* reduces its velocity, radius and pitch to very small values, but increases its angular velocity in response to introduction of *Storeatula major*, changes that seem to reduce its hydrodynamic signature while still scanning its environment as "a spinning antenna". Conversely, the fast *P. piscicida* increases its speed (to 280-340 $\mu\text{m s}^{-1}$), radius and angular velocity, but slightly reduces its pitch when *Rhodomonas* sp is introduced, suggesting preferred predation tactics of an "active hunter". Statistics of NND show that organisms, predators and prey, remain randomly distributed relative to their own species, but in the predator-prey mixture there is statistically robust evidence of predators clustering around their preys.

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FINE SCALE DISTRIBUTION AND ABUNDANCE OF LARGE AND SMALL PHYTOPLANKTON IN MONTEREY BAY, CA

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In turbulent coastal ocean waters, phytoplankton abundance and community composition can vary dramatically over small spatial and temporal scales. As part of the 'Layered Organization in the Coastal Ocean' program, we have examined the fine scale distribution, abundance and characteristics of both small picophytoplankton and large microphytoplankton in Monterey Bay, CA during August 2005 and July 2006. Picophytoplankton cell density, cell size and fluorescence characteristics were determined with image analysis. Microphytoplankton cell density and taxonomic composition were determined with light microscopy. The vertical cell density distributions of different size classes were sometimes similar and at other times very different. Dissimilar patterns of distribution suggest that certain taxa or groups have unique functional characteristics and occupy different ecological niches. Their relative distribution, abundance and population dynamics may influence ecosystem structure and function.

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CHRYSOPHYTES AND DIATOMS FROM AN EOCENE ARCTIC LAKE: IMPLICATIONS FOR BIOGEOGRAPHY, AND EVOLUTIONARY STASIS

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A drill core raised from the Giraffe kimberlite pipe in Canada's Northwest Territories contains 67 m of stratified nonmarine sediment of Middle Eocene age (Lutetian; 42-48 Ma). These sediments were deposited post-eruptively within the diatreme maar lake, and thus grade from deep lake to peatland facies as the basin progressively infilled. Despite being situated near the Arctic Circle, the lake existed during the Cenozoic hot house and the region was subtropical. We have recorded abundant numbers of chrysophyte remains (cysts, scales and bristles) and diatom microfossils from many sections of the core, all remarkably preserved. Scale remains from the genera *Mallomonas*, *Synura*, *Chrysospharella* and *Spiniferomonas*, representing at least 20 different species, have been documented and represent the oldest records for these genera. Remains of diatoms representing the Stephanodiscaceae, Aulacoseiraceae, Eunotiaceae, Fragilariaceae, and Naviculaceae have also been observed, many of which push back the age of specific genera and lend insights on the evolution of specific structures. Some of the taxa have not been reported in modern-day studies and presumably represent new and probably extinct species. Others are astonishingly similar to extant species and differ only slightly from their modern counterparts. These observations imply that the scaled chrysophytes and diatoms have experienced prolonged intervals of evolutionary stasis during Neogene and Quaternary times. Among the fossil remains in this Arctic lake are ones known today only from warm subtropical and tropical localities.

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GREAT LAKES DIATOM TOOLS: ADVANTAGES OVER CHEMICAL MEASUREMENTS IN PALEOLIMNOLOGICAL AND MONITORING PROGRAMS

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Diatoms are known to be powerful indicators of environmental change, and so their indicator properties have been characterized for use in coastlines of the Laurentian Great Lakes. Our first aim in this study was to identify the responses of coastal diatoms to environmental variables, and identify gradients that would be of interest to lake managers. More than 2,000 diatom taxa were identified from sites along the American coastlines of all five Great Lakes. The relationship between diatoms and limnological parameters was explored using multivariate analysis. Stressor variables that explained variation in the diatoms included nutrients, solids and chloride, which are proxies for eutrophication, erosion and salinification, respectively. A diatom inference model for nutrients provided a robust reconstructive relationship, reflecting the strong response of the diatoms to disturbance and the promise for diatom-based applications in coastal ecosystems. Furthermore, relationships between watershed stressor data (e.g., agricultural and urban development) and diatom-inferred water quality

indicated that diatom-inferred data were superior to water quality data derived from direct measurements. These links to anthropogenic impacts in Great Lakes watersheds indicate the advantages of including algal-based approaches in monitoring programs and paleoecological assessments. Robust tools are now available to agencies and managers concerned with environmental disturbance in Great Lakes coastlines.

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LINKING THE PRESENT AND THE PAST USING CHRYSOPHYTE DNA SEQUENCES - NEW PERSPECTIVES FOR CYST-BASED RECONSTRUCTIONS

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Chrysophytes (Chrysophyceae and Synurophyceae) produce siliceous resting cysts (stomatocysts) that are widely used as indicators of past environmental conditions. These ornate stomatocysts are rarely linked to species, which are described using vegetative cell morphology. Stomatocysts are therefore classified using an artificial system based upon cyst morphotypes. This causes taxonomic uncertainty and, hence, errors in cyst-based reconstructions. Furthermore, the ecological information about species cannot be used in paleoecological studies. DNA analysis has become increasingly important for chrysophyte systematics, and species taxonomy based on DNA sequences agrees with traditional morphological species concepts. Our objective was to link stomatocysts to the species that produce them by using DNA sequences from both, providing a means for (i) natural classification of stomatocysts, and (ii) incorporating modern ecological knowledge in paleoenvironmental studies. We picked single stomatocysts from sediment-trap samples and amplified SSU rRNA and rbcL genes by polymerase chain reaction (PCR). The genes were sequenced, aligned with existing sequences from identified species and analyzed using phylogenetic analyses. The cysts remained intact during PCR amplification, and cysts were removed and identified by environmental scanning electron microscopy (ESEM). Stomatocysts with orphan sequences, i.e., sequences from unknown species, were given an approximate identification by their position on phylogenetic trees. As sequences become known for more species, orphan sequences from unidentified cysts can be assigned to a species without further laboratory work. Our approach bridges the gap between modern ecology and paleoecology as well as between modern systematics and fossil cysts. These provide new perspectives for improving cyst-based paleoenvironmental reconstructions.

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ENIGMATIC REPRODUCTIVE STRUCTURES IN *PLATYSIPHON VERTICELLATUS* WILCE (1962): AN ARCTIC ANNUAL ENDEMIC

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Platysiphon verticellatus is a rare thalloid brown alga. We cite its known occurrence from northeast and northwest Greenland and northern Baffin Island, its mode of thallus development, portions of its life history and its distinctive annual seasonality. Descriptions are based on seven years of in situ studies in northern Baffin Island, Nunavik, Canada, and culture observations. Despite collections from the entire light season, unilocular and plurilocular sporangia remain unknown. In culture, thalli become reproductive after varying periods of darkness at temperature from 4-8 °C. Vegetative cells with numerous discoidal plastids and photosynthetic reserves change dramatically as the protoplast shrinks from the cell wall to become spherical, ultimately irregular in outline. Cytoplasmic change occurs in cells of the median portion of the blade. Protoplasts attach to the inner face of the parent cell walls where discharge pores are formed on either side of the thallus, probably by enzymatic wall digestion. Protoplasts emerge and attach to the surface of the thallus. A pad of “cement” is then apparent between the protoplast and parent cell wall. Protoplasts form a cell wall, resulting in a novel, rigid, lightly sculptured, cyst-like resting stage. Cysts contain one protoplast surrounded by a membrane. Cysts form an aperture under low light that becomes irregularly ruptured to permit release of a biflagellate zoospore. Zoospores have a parietal plastid, one long anterior flagellum and one short posterior flagellum, and lack an eyespot. They attach to solid substrata and initiate branched filaments. Cells of the filaments contain non-pyrenoidal plastids. Ordinal status of *Platysiphon* remains questionable. Novel morphological and reproductive features suggest a new family Platysiphonaceae, alternatively, genome sequence analysis likely points to a second genus in the Halosiphonaceae.

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BARCODING BROWN ALGAE: THE DNA BARCODE INITIATIVE IS CHANGING OUR VIEW OF THE PHAEOPHYCEAE IN CANADA

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Historically, the process of discovering new species involved finding a unique specimen in the field, taking it back to the lab for further examination and determining if it was significantly different than other known species. However, by screening only a small number of obviously unique collections there is a high probability of overlooking cryptic diversity. For the past year and a half we have been scaling up our field collections as part of the Canadian Barcode of Life Network initiative, which aims to identify every eukaryotic species in the country and their corresponding distribution. In order to screen this large number of collections we are utilizing the DNA barcode (the 5' end of the cytochrome oxidase 1 gene) to quickly assign the collections to a species. The barcode has been successful in differentiating between the majority of brown algal species, however, in cases where the barcode fails to clearly separate species we are given the opportunity to explore some very exciting science. By screening a large number of samples from across Canada we are learning about species biogeography, incipient speciation, as well as cryptic (and not so cryptic) diversity. Here we present several of the stories we are currently working on in the Phaeophyceae, including: the importance of sequencing sufficient isolates, competitive exclusion of species and hybridization and the common occurrence of cryptic species from the smallest filaments to the grand kelps. We consider that, far from being the ruin of taxonomy envisioned by critics, the barcode is uncovering a plethora of fascinating stories that will keep taxonomists busy for years to come.

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PHYLOGENETIC RECONSTRUCTION OF THE ORDER SURIRELLALES (BACILLARIOPHYTA) FROM MORPHOLOGICAL AND DNA SEQUENCE DATA

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The order Surirellales currently consists of 9 genera in 3 families: Entomoneidaceae, Auriculaceae, and Surirellaceae. Surirellales species exhibit diverse valve morphologies and are considered the most “advanced” of the pennate diatoms due to possession of canal raphe systems and elaborate keels. Although Surirellales monophyly is supported by both morphological and molecular data, the phylogenetic relationships at the family level and below are uncertain. As part of a large-scale phylogenetic study of Surirellales, morphological characters and DNA sequence data from the nuclear, chloroplast, and mitochondrial genomes are being gathered. We present preliminary estimates of Surirellales phylogeny from these datasets, analyzed individually and in combination. Monophyly of the families and major genera, and phylogenetic congruence between morphological and DNA sequence data, are evaluated and discussed.

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DIFFERENTIAL OCCURRENCE OF LAND PLANT EXTRACELLULAR POLYSACCHARIDES IN THE CHAROPHYCEAN GREEN ALGAE AND IMPLICATIONS FOR PLANT EVOLUTION

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Charophycean green algae (Streptophyta, Viridiplantae) (CGA) represent a diverse assemblage of taxa that are ancestral to land plants. The morphological forms displayed within this group are, in part, defined by their cell walls. Recent investigations of biochemistry and development of charophycean cell walls have shown that many cell wall polymers of higher plants are also found in charophycean green algae. We have recently initiated a comprehensive analysis of the cell walls of select CGA using Comprehensive Microarray Polymer Profiling (CoMPP), immunocytochemical labeling and direct biochemical analyses. Homogalacturonic acid-

based polymers are found throughout the charophycean group including the cell walls of *Chlorokybus atmophyticus*, the primary walls of Zygnematalean taxa, the polar tips of young branch cells of *Coleochaete nitellarum* and *Chara corallina*, the hair cells of *C. nitellarum* and the inter-scale fibrillar matrix of *Mesostigma viride*. β (1-4)-mannans are found in the walls of *C. corallina* while non-fucosylated xyloglucans are found only in the walls of *C. corallina* and *Spirogyra* sp. Arabinogalactan proteins are found associated with the outer region of the pore complexes of placoderm desmids and extensin-like epitopes are found throughout the charophycean group. These results suggest that many of the cell wall polymers found in terrestrial plant walls evolved before the emergence of green plants onto land and provide the basis for future molecular investigations leading to an understanding of the evolutionary trends leading to complex morphological CGA forms and ultimately, land plants.

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THE GREEN SYMBIONTS OF *ANTHOPLEURA* FORM A DISTINCT MONOPHYLETIC TAXON IN TREBOUXIOPHYCEAE (CHLOROPHYTA)

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The terms zoochlorellae and zoochlorella are used commonly to refer to the green algal symbionts of invertebrates and protists. The term was first used to describe the green algal symbionts of freshwater hydras. The green algae known to symbiose with the Pacific sea anemones *Anthopleura elegantissima* and *A. xanthogrammica* also have been called zoochlorellae. Ecological work on *Anthopleura* and their symbionts has been extensive, whereas the taxonomic placement of their green algal symbiont has remained largely untouched until recently. The limited and somewhat cryptic morphology of the *Anthopleura* green symbionts, along with their inability to be cultured, has contributed to a delay in taxonomic description. In order to determine the taxonomic placement of *Anthopleura* zoochlorellae we have gathered morphological and molecular data from samples of *A. xanthogrammica* and *A. elegantissima* tissue containing green symbionts from their known range along the North Pacific coast, including Alaska, Oregon and Washington, USA. TEM is being used to determine how cell division occurs in zoochlorellae. The cell wall morphology of the *Anthopleura* zoochlorellae is being examined using SEM to elicited characters that may help distinguish zoochlorellae from its algal relatives. Our molecular phylogenetic analyses of nuclear 18S rDNA, and chloroplast *rbcL* shows monophyly of all green symbionts isolated directly from the anemones, regardless of the host species or geographic location. The *Anthopleura* symbionts share a close relationship to *Elliptochloris* (Trebouxiophyceae). We are working to determine if the anemone symbionts are a species of *Elliptochloris* or a sister genus.

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TROPICAL SUBAERIAL MICROCHLOROPHYTES AND THE QUEST FOR LAND

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Subaerial microchlorophytes are microscopic green algae living on exposed surfaces above the soil. They are perhaps the most obvious, and yet most overlooked, group of algae. Their economic impact is significant in biodeterioration events, citric cultivars, bioindicators, and biotech applications. The biodiversity of subaerial microchlorophytes is typically underestimated; this is particularly problematic in tropical rainforests, for which this flora is poorly known and in urgent need of further investigation. Our research efforts are targeting the biodiversity and evolutionary history of subaerial microchlorophytes, in particular from tropical rainforests. Molecular phylogenetic analyses of nuclear and chloroplast sequences from our ulvophycean isolates (Trentepohliales and Cladophorales orders) indicate that at least two strictly subaerial microchlorophytes groups have a marine origin. Subaerial microchlorophytes may represent an example of morphological convergence, with distant or unrelated taxa converging on a limited number of thalli types. Other biochemical and reproductive subaerial adaptations are also notable. Several green algal groups have conquered terrestrial habitats: Charophycean, Trebouxiophycean, and Chlorophycean subaerial microchlorophytes made attempts to colonize the land via freshwater habitats. Our phylogenetic reconstructions suggest that the marine ulvophyceans also made this conquest at least twice. The present subaerial ulvophytes (with marine ancestors) made the transition to land via a shortcut, that is, bypassing freshwater habitats and following a more direct

“invasion strategy.” Tropical subaerial microchlorophytes may provide the keys to understand the struggles and solutions that primordial photosynthetic forms went through in order to conquer the land and become the most notable feature of our planet.

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DIVERSITY, EVOLUTION AND ECOLOGY OF THE TRENTEPOHLIALES (ULVOPHYCEAE, CHLOROPHYTA) IN TROPICAL REGIONS

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The ulvophycean order Trentepohliales is one of the most widespread groups of subaerial green algae. Although present also in temperate areas, these algae are usually reported as most abundant and diverse in the tropics. Remarkably, however, very few studies have investigated the diversity of the Trentepohliales in tropical regions; India, Queensland and the area of Bogor (Java, Indonesia) are the only regions for which detailed studies are available. We are currently investigating the diversity of this group in the tropics by a combination of morphological studies and analysis of sequences of the SSU rRNA, LSU rRNA and *rbcL* genes. Recent surveys conducted in French Guiana and Panama have revealed a high species diversity of this order; six species have been newly recorded for the Americas and two new species have been described. A marked ecological differentiation has been observed in these regions: whereas some species are strictly associated with rainforest habitats, others prefer more exposed habitats and are frequently found on artificial substrata. The results of the molecular analyses so far suggest that the SSU rRNA gene is suitable for phylogenetic inference in this group. Phylogenetic analyses on the *rbcL* gene indicate that in the Trentepohliales this marker has evolved faster than SSU rRNA, and its systematic value in this order is currently being assessed.

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THE SEARCH FOR CLASS AND ORDINAL LEVEL SYNAPOMORPHIES IN *SPONGIOCHRYSIS HAWAIIENSIS* (ULVOPHYCEAE, CHLOROPHYTA)

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Previous molecular based phylogenetic analyses placed the newly discovered subaerial green alga *Spongiochrysis hawaiiensis* into class Ulvophyceae and more specifically into the order Cladophorales (Rindi *et al.* 2006). Although molecular based phylogenies have clearly placed *Spongiochrysis* in the Cladophorales and the Ulvophyceae, the alga exhibits many autapomorphic or unique morphological traits at both the class and ordinal levels. It is the only member of the class to reproduce via a budding-like mechanism. This reproductive mechanism appears to be very similar to those found in the Trebouxiophytes *Marvania* and *Stichococcus*. At the ordinal level, *Spongiochrysis*' golden colored plastid, subaerial growth habitat, and coccoid cell shape make it unique to Cladophorales. However, it must be noted that *Spongiochrysis* lacks any shared morphological character state at the ordinal level. Our current maximum parsimony and Bayesian inference analyses of a more taxonomically robust SSU rDNA dataset (which includes all known ulvophycean orders) strongly supports the alga's cladophoralean affinity. Additionally, SEM and TEM micrographs are being used to search for morphological synapomorphies that may provide insight on the character evolution of this novel and enigmatic subaerial microchlorophyte.

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INVESTIGATIONS ON THE SUBAERIAL TRENTEPOHLIALES (ULVOPHYCEAE, CHLOROPHYTA) IN THE SOUTHEASTERN USA

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The Trentepohliales, being neither aquatic nor terrestrial but subaerial, are a unique, understudied, and often overlooked group of green algae found throughout the Southeastern USA and in many habitats of high humidity worldwide. *Cephaleuros*, a genus within the Trentepohliales, requires special attention. The generitype, *C. virescens*, was originally described as a fungus. This taxon has been reported in the literature from nearly every tropical and subtropical region, including the Southeastern USA. Important aspects of their

unique habitat requirements and the current morphology-based classification in *C. virescens* combine to present rather unique challenges to the researcher. In order to assess the systematic circumscription and widespread distribution of *C. virescens*, samples of this taxon have been gathered from the Southeastern USA and overseas. The use of primers specifically designed for the chloroplast-encoded *rbcL* from *Cephaleuros* have been successful in amplifying sequences for phylogenetic analyses. Present results from this study indicate that several entities representing different lineages are currently grouped under the name “*Cephaleuros virescens*”. These taxa share a similar morphology and habitat and may represent a case of morphological convergence. Based on topotype material from the Guiana region, *C. virescens* appears to be restricted to the tropical Central and South America, with several unnamed species occurring in Southeastern USA and overseas. Morphological analyses involving cytokinesis type are in progress to enhance the characterization of these taxa. Samples of the Trentepohliales have been gathered, preserved, and cultured from the Southeastern USA and used for a thorough morphological and molecular investigation to reveal the diversity and phylogenetic relationships of the trentepohlian taxa distributed within the Southeastern USA.

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EFFECTS OF *KARLODINIUM VENEFICUM* ON EARLY LIFE HISTORY STAGES OF THE EASTERN OYSTER

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The bloom-forming dinoflagellate, *Karlodinium veneficum*, can have detrimental effects on marine life, but little was known about its effects on early life history stages of bivalves. In the Chesapeake Bay region, blooms of *Karlodinium* over-lap with the spawning season of the eastern oyster, *Crassostrea virginica*. In laboratory experiments, we determined the effects *K. veneficum* on the survival and development of embryos and larvae of the eastern oyster. At $\sim 2 \times 10^4$ cells ml⁻¹, *K. veneficum* (strain CCMP 1974) caused significant mortality to oyster embryos within 1 day and almost no embryos developed into D-hinge larvae. This effect was not alleviated by the provision of an alternate food source (Isochrysis). Significant mortality was observed when larvae were exposed to *K. veneficum* at concentrations as low as 2.5×10^3 cells ml⁻¹. A two day exposure of larvae to 5×10^3 cells ml⁻¹ of *K. veneficum* reduced yield of mature larvae by $\sim 66\%$. The *K. veneficum* cultures that we used in our experiments were relatively low in toxin content, more toxic strains could be expected to cause mortality at lower cell concentrations. Survival and maturation of larvae may be reduced when spawns of the eastern oyster coincide with high bloom densities of *K. veneficum*.

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DEVELOPMENT OF A TOXIC DINOFLAGELLATE (*KARLODINIUM VENEFICUM*) BLOOM IN A SHALLOW, EUTROPHIC, LAGOONAL ESTUARY

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A dense bloom of the ichthyotoxic dinoflagellate *Karlodinium veneficum* was discovered in the Neuse River Estuary, NC on 19 October, 2006 and was associated with four subsequent fish kills. This bloom was fostered by runoff following tropical storm Ernesto which input particulate and dissolved nutrients. Initially runoff lead to increased flushing, light limited productivity and low algal biomass. As riverine discharge declined and the water column stabilized, it established hydrodynamic conditions favorable for the development of a surface frontal zone where lower dispersion rates were favorable for growth. During this period a prolonged period of low wind further allowed vertical stratification and development of hypoxic bottom conditions that produced the highest hypolimnetic concentrations of remineralized NH₄⁺ ever measured in the estuary. A brief wind event mixed regenerated nutrients throughout the water column. A continued period of stable runoff, calm winds and a highly stratified water column provided salinity, light, nutrient and hydrologic conditions ideal for phytoplankton growth. The resultant community became dominated by dinoflagellates, the most successful of which was the mixotroph *K. veneficum* ($>200,000$ cells ml⁻¹). The success of this species is probably due to its ability to produce the karlotoxin KmTx2, which aids in the capture of algal prey during mixotrophic grazing and in deterring microzooplankton grazers. Once the bloom was established, small-scale estuarine physical

processes coupled with vertical migration behaviors acted to further concentrate *K. veneficum* cells. The bloom demise was linked to disruption of an already senescing population by a turbulent wind mixing event. Toxin released from these cells was postulated to be the cause of the concurrent fish kills. Data that support this assumption include the likely movement of the disrupted bloom into the fish kill area, the presence of *K. veneficum* at the kill sites, and the characteristic premortem symptoms of karlotoxin poisoning which include air gulping and lethargy despite high ambient DO conditions.

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GRAZING ON THE ESTUARINE BLOOM FORMING SPECIES *KARLODINIUM VENEFICUM* DETERRED BY PRODUCTION OF KARLOTOXINS

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Karlodinium veneficum is a ubiquitous mixotrophic dinoflagellate common to estuaries and coastal regions around the world. While normally present at relatively low concentrations, this species produces karlotoxins and is capable of forming dense “mahogany tides” which kill fish when nutrient inputs are high and other environmental conditions favorable. During the development of these blooms, *Karlodinium veneficum* often outcompetes other dinoflagellates as these blooms develop. Recent evidence suggests this may be due to the karlotoxins selectively reduction of microzooplankton grazing, particularly by *Oxyhris marina*, a major predator of *K. veneficum*. In this study we examined the ability of karlotoxins to deter grazing of a dominant estuarine macrozooplankton species, *Acartia tonsa* (Calanoidia: Copepoda). Specifically, *A. tonsa* grazing rates were determined for both toxic and non-toxic *K. veneficum* strains and various proportions of mixed toxic and non-toxic strains. Staining with CellTracker™ Green CMFDA was used to differentiate one strain of *K. veneficum* from the other in final counts. The results showed that the toxic strain reduced grazing of *K. veneficum* relative to the non-toxic strain by ~50%. In the mixtures of toxic and non-toxic cells, grazing was reduced in proportion with the abundance of toxic cells. Examination of the stained cells indicated that grazing rates were reduced on both the toxic and non-toxic strains. This indicates that *A. tonsa*'s ability to graze was compromised. However, *A. tonsa* lacked the ability to identify or select for or against the toxic and non-toxic cells. The significance of these findings on the development of harmful algal blooms will be discussed.

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STRAIN VARIABILITY IN *KARLODINIUM VENEFICUM* (DINOPHYCEAE)

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Karlodinium veneficum is a potentially ichthyotoxic bloom forming dinoflagellate found in estuarine systems throughout the world. A culture collection from the U. S. Atlantic coast (25 unialgal strains) was used to identify genetic correlates of karlotoxin types in *K. veneficum*. All strains had identical ITS sequences and pigment profiles. Differences in genotype were determined using DNA sequences isolated from a library of enriched repeat sequences, mitochondrial cytochrome b, cytochrome c oxidase I, and genome size. One marker, named F4 yields a 485 bp fragment for U.S. Chesapeake Bay strains producing karlotoxin 1 while the same locus yields a 283 bp fragment for the other U.S. strains producing karlotoxin 2. In some strains, both *cob* and *cox1* primers yielded one single sequence whereas in others numerous sequences highly recombined with other genes. Genome size varied significantly among strains, ranging 5-16 pg cell⁻¹. These results suggest that *K. veneficum* populations comprises diverse genotypes that may not be detected by ITS and that some of the markers we analyzed are a promising tool for application to field samples to differentiate distinct populations.

CRYPTOPHYTES DRIVE BLOOMS OF MIXOTROPHIC HARMFUL ALGAE: A TESTABLE HYPOTHESIS BASED ON *KARLODINIUM VENEFICUM* IN CHESAPEAKE BAY

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We hypothesize that prey abundance, particularly nanoplanktonic (<20 microns) cryptophytes, drives the formation of mixotrophic harmful algal (HA) blooms in eutrophic environments. Cryptophytes represent a substantial portion of phytoplankton biomass in eutrophic coastal estuaries such as Chesapeake Bay where their high growth rates at low light conditions allow them to become 'first responders' to episodes of new nutrient input. *Karlodinium veneficum* is frequently present in such systems at sub-bloom concentrations, although transient blooms of *K. veneficum* tend to occur in highly eutrophic environments. *Karlodinium veneficum* grows faster when cryptophytes are available and produces an allelochemical, karlotoxin (KmTX), that allows it to effectively compete for cryptophytes, even in the presence of another heterotrophic predator, *Oxyrrhis marina*. Several studies show correlations between cryptophytes and dinoflagellates *in situ*, broadly suggesting a predator-prey relationship between these two functional groups. We will attempt to forecast *K. veneficum* blooms by using phycoerythrin fluorometers on Maryland Department of Natural Resources real-time continuous monitoring platforms to detect potential prey populations in locations where *K. veneficum* blooms have occurred annually for the last two years (Corsica R., MD). We will support our continuous monitoring effort with microscopic and molecular characterization of *in situ* cryptophyte and dinoflagellate populations, and laboratory studies of the grazing and growth efficiency of *K. veneficum* on cryptophyte species representative of the functional and phylogenetic diversity that is likely to be present *in situ*.

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PRYMNESIUM IN FLORIDA: AN EMERGING HARMFUL ALGAE PROBLEM

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Harmful algal blooms (HABs) can cause mass animal mortalities, shellfish and finfish intoxication, and neurological disorders in mammals. HABs have been associated with animal mortalities in a variety of aquatic systems worldwide. In Florida waters more than 60 species of harmful microalgae have been documented. Historically, many fish kills have been attributed to low dissolved oxygen levels but closer examinations of these events often indicate that ichthyotoxic microalgae may play a more prominent role. Finfish and shellfish exposed to microalgal toxins can experience physiological dysfunction, impaired feeding and respiration, pathological abnormalities, or at lethal levels, mortality. Historically, mortality and illness events in Florida have been associated with blooms of the dinoflagellate species, *Karenia* spp., *Kryptoperidinium foliaceum*, *Pyrodinium bahamense*, *Alexandrium monilatum*, and *Takayama* spp., the raphidophytes, *Chattonella subsalsa* and *Heterosigma akashiwo*, or the cyanophytes, *Microcystis aeruginosa*, *Anabaena circinalis*, and *Cylindrospermopsis raciborskii*. However, in the past three years we have seen an increase in fish kills associated with blooms of the prymnesiophyte, *Prymnesium*. Globally, five *Prymnesium* species were reported to be ichthyotoxic, with the majority of fish kill reports involving *P. parvum*. *Prymnesium* species produce hemolytic and ichthyotoxic compounds (prymnesin-1 and prymnesin-2). Prymnesins are released into the water during bloom events and fish are directly affected as these hemolytic and ichthyotoxic compounds are absorbed by the gills. Other aquatic organisms also appear to be susceptible to prymnesins. Globally, *Prymnesium* blooms have caused significant economical impacts to aquaculture. Recently, *Prymnesium* blooms in Texas have become a significant problem for recreational fisheries. To date, blooms of *Prymnesium* in Florida have only occurred in non-commercial ponds, but nevertheless have impacted recreational facilities. Here we report on the initial mortality events in Florida that are related to blooms of *Prymnesium parvum* and *P. saltans*.

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TRACHELOMONAS IN IOWA WETLAND MITIGATION SITES

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The euglenoid *Trachelomonas* was observed as a part of an ongoing study of Iowa wetland mitigation sites. *Trachelomonas* diversity can be used as an indicator of wetland habitat quality, having a large number of species characteristic of shallow soft-bottomed water bodies. A total of 29 taxa were observed, and of these 19 were identified and matched with existing classifications based on visual characteristics. Multiple samples from 11 mitigation sites were observed and compared with samples from three natural wetlands, wherein the most diverse site contained 15 different taxa of *Trachelomonas*, and the least diverse site held two taxa. The most varied individual sample included seven distinct taxa. Data on the number of *Trachelomonas* species per wetland mitigation site suggests that some locales are supporting a diverse array of life, while others may have a limited amount of biodiversity based on the *Trachelomonas* diversity.

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ROLE OF ALGAE IN ASSESSING AQUATIC ECOLOGICAL CONDITIONS OF WADEABLE STREAMS FOR USGS NAWQA SURFACE WATER STATUS AND TRENDS

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The U.S. Geological Survey National Water-Quality Assessment (NAWQA) Program provides national-scale perspective and assessments on aquatic biology and the chemical and physical factors that affect ecological condition. Since 1991, data have been collected by NAWQA on chemistry, hydrology, land use, stream habitat, and aquatic life in nearly all 50 states with a nationally consistent study design and uniform methods of sampling. The data are being used to assess the status and trends of aquatic ecological conditions (invertebrates, fish, algae, and habitat) in rivers and wadeable streams and to develop key ecological indicators of aquatic health. Algal-community data for more than 5,000 taxa from over 1,500 sites have been identified, reviewed, and analyzed to assess stream-water quality and ecological relevance throughout the United States. The data are available at <http://water.usgs.gov/nawqa/data.html>, along with chemical, habitat, and land cover data associated with the algal samples. Algal optima and tolerance values for conductivity, ionic composition, pH, and nutrient information have been used to create algal metrics. For ecological status and trends analysis, 58 sites were selected (465 quantitative samples, an average of 8 per site). Algal metrics were able to detect site differences between the 23 reference sites and sites influenced by human activities (15 agricultural, 20 urban). Case studies illustrate how algae data expands interpretation from speculation based on the invertebrate and fish communities to a more complete explanation using multiple lines of evidence. Specifically, algae augment our understanding of nutrient dynamics (trophic interactions) in streams, and show trends in conditions at sites over time. Interpretation of water-quality is strengthened by algae data which contribute to the multiple lines of evidence. Routine collections of algal samples also yield data on the occurrence of potential taste-and-odor problems and toxic blue-green algae, such as *Microcystis aeruginosa*, and *Anabaena* spp., as well as early detection of nuisance and exotic species, such as *Didymosphenia geminata*.

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CHARACTERIZATION OF A ROCKY INTERTIDAL SHORELINE WITHIN ACADIA NATIONAL PARK: IMPACT OF SHORT-TERM TRAMPLING AND IMPLICATIONS FOR MANAGEMENT

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Millions of people visit Acadia National Park (ANP) in Maine each year. For example, over six million people visited ANP between 2004 and 2006, with the peak period being July - September. In July 2002, ANP staff received management responsibilities from the National Park Service (NPS) for a 2 km section of shoreline and approximately 100 acres of land located on Schoodic Point, Winter Harbor, Maine. Prior to July 2002, this land and shoreline were a U.S. Naval Base. Due to its military status, there was no public access to this shore

from the mid-1930s until its transfer to the NPS in 2002. The restricted access protected the shore, which appears to be unusually pristine. Studies elsewhere have found an inverse relationship between the intensity of foot traffic on rocky intertidal shores and the percent cover of macroalgae and abundance of large, sessile organisms. In summer 2006, we conducted an observational pilot study to determine what visitors to ANP do in the intertidal zone and how much time they spend in the intertidal zone. We observed higher numbers of visitors at sites with designated pull-out areas and easy access to the shoreline. Results of the first visitor survey, conducted in May 2007, were very similar to those observed during the pilot study. In addition, we will characterize the biota on the shoreline and conduct an experimental trampling study. Six replicates of three plots each were randomly assigned to one of three treatments (Control, Low, or High) and the first set of treatments applied during May 2007. Each trampling treatment will be applied once a month through October 2007. Characterization of the shoreline will provide the NPS with a baseline on the assemblage structure at five study sites with similar exposures in ANP, and results of the trampling study will provide data on the potential impact from visitor use. The Park Service will use these data in management of the shores. (Funded by the NPS).

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THE EFFECTS OF WATER PHOSPHATE CONCENTRATIONS ON THE DISTRIBUTION AND DEGREE OF CALCIFICATION OF TWO CALCAREOUS GREEN MACROALGAE

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Calcification among the algae plays important roles in defense against destruction by herbivores and wave damage. In tropical marine habitats, calcified species of the green algae order Bryosidales are important CO₂ sinks and sediment producers. Though extensive studies have been performed on the ecological and physiological aspects of the calcification process in these algae, few have measured responses to increased nutrient loads, which is essential to know since eutrophication has become a problem for tropical reefs, a common habitat for these algae. The percent calcification of *Halimeda incrassata* (Ellis) Lamouroux and *Penicillus capitatus* Lamarck were compared in phosphate-rich versus phosphate-limiting Florida coasts. Calcareous algae from the Florida Keys were significantly more calcified than those in Tarpon Springs ($p < 0.001$). Differences in water column nutrient concentrations were analyzed and compared to differences in calcification. Nitrogen (Nitrate + Nitrite and Ammonium) levels were similar in both habitats. Phosphate and silicate concentrations, however, were much higher in Tarpon Springs than in the Keys, suggesting that one, or both, of these nutrients significantly inhibits macroalgal calcification. Experimentally phosphate enriched plots led to a significantly decreased ($p = 0.017$) mean percent calcification in new growth compared to control plots at the same site. The greater distribution of calcareous macroalgae in the Keys further supports the hypothesis that increased phosphate results in decreased calcification, as calcification is necessary as both an anti-grazing defense and as a structural support component. Tampa Bay, which is adjacent to Tarpon Springs, lacks calcified green macroalgae, but has an abundance of fleshy relatives. Abnormally high phosphate levels recorded in Tampa Bay, presumably due to phosphate mining, suggest that calcified green macroalgae cannot proliferate in areas with high phosphate levels. Transplant experiments are in progress to confirm this hypothesis.

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A REPORT ON POSTAGE STAMPS DEPICTING ALGAE

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So-called topical stamp collecting has been a popular pursuit for many philatelists. In recent years there has been a growing number of postage stamps that depict algae, and so the fields of phycology and philately have come together. Now there are a few dozen countries that have included algae either as primary objects or as background material. In some instances sets of stamps have been issued showing a number of algae. Most often marine macro-algae have been the focus of attention, but there have also been freshwater algae, such as the *Cladophora* balls ["marimo"] of Japan, and also examples of Cyanobacteria, planktonic diatoms, and snow diatoms. Brown seaweeds such as *Macrocystis* and *Durvillaea* have often been portrayed on stamps, especially by countries of the southern hemisphere. There has also been frequent depiction of marine algae that have economic value or of the actual gathering of these seaweeds. Sets of stamps exclusively featuring algae have

been issued by Algeria, Fiji, the Faroes, Mozambique, Senegal, South Africa, Tristan da Cunha, and Yugoslavia. In other cases, some countries, such as New Zealand, the Philippines, and Chile, have issued sets of stamps devoted to marine life, and these have included stamps with seaweeds. The Falkland Islands is noteworthy in having issued multiple stamps featuring the algae occurring there, as well as the early French explorers Lesson and Dumont d'Urville with their namesake algal genera *Lessonia* and *Durvillaea*.

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DESMID-RICH BIOFILMS AND HETEROTROPHIC PROTIST ASSEMBLAGES OF ADIRONDACK WETLANDS

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Benthic habitats of the photic zones of shallow wetlands of the northern hemisphere are typically characterized by a rich assemblage of photosynthetic microorganisms including green algae and diatoms. Primary productivity by wetland algae has been shown to rival and sometimes surpass that of aquatic macrophytes and the flow of algal-derived photosynthate may contribute significantly to food chains, nutrient sequestering and substrate/biofilm stabilization. Most benthic wetland algae reside in complex and dynamic microbial communities or biofilms that are attached to various substrates. During a long-term study of biofilms of an oligotrophic wetland in the Kayaderoseros watershed of the southeastern region of the Adirondack region of New York (USA), desmids (Streptophyta, Viridiplantae) constituted a significant percentage of the algal flora. These green algae represented significant colonizers of artificial substrates seeded in the wetland during the summer months and made up to nearly 25% of the algal population of older, 8-28 day-old biofilms. Desmid numbers were highest in elevated water temperatures, high light and low phosphate, nitrate and silica levels. Many of the desmids secreted prodigious amounts of an extracellular polymeric substance or EPS that contributed to the biofilm matrix and harbored a large and diverse number of heterotrophic microbes. The distribution of bacteria in young biofilms was fairly uniform but after 4-8 days post-colonization, noticeable patchiness was observed. Heterotrophic protists, including ciliates and numerous non-testate amoebae, were observed in the biofilms. The density and carbon content of amoebae were assessed in 10-day and 21-day biofilms since amoebae are known to penetrate deeply into organic floc and biofilms. The ratio of the density of amoebae (c. 103/cm³) to desmids in the biofilm was substantial in the range of 70%.

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EFFECTS OF LIGHT INTENSITY AND PREY CONCENTRATION ON GROWTH, GRAZING, AND SURVIVAL OF THE MIXOTROPHIC DINOFLAGELLATE *DINOPHYSIS ACUMINATA*

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The marine dinoflagellate *Dinophysis acuminata* has been formally known as a photosynthetic species containing cryptophyte-like plastids, but recently the feeding mechanism and its phagotrophy on the ciliate *Myrionecta rubra* were revealed clearly. Using culture of *D. acuminata*, we examined the effects of light intensity and prey concentration on growth and ingestion rates of *D. acuminata* when fed on the prey *M. rubra*. While mixotrophic growth rate of *D. acuminata* increased sharply with increasing a mean prey concentration (at level of <1000 cells ml⁻¹) and reached a maximum growth rate (μ_{max}) of 1.24 d⁻¹ at saturating mean prey concentration of approximately 2000 cell ml⁻¹ under 20°C and continuous illumination of 60 $\mu\text{mol m}^{-2} \text{s}^{-1}$, phototrophic growth rate of *D. acuminata* cultures in the absence of prey was 0.15 d⁻¹ under the same culture condition. After depletion of prey, *D. acuminata* cells underwent 1 or 2 cell divisions and then entered at stationary phase. The growth rates of *D. acuminata* with and without prey (i.e. the mixotrophic and phototrophic growth, respectively) increased with light intensity between 0 to 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$, while the ingestion rates appeared not to be greatly susceptible to light intensity, except for that at 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at which relatively higher ingestion rate was observed. In dark, *D. acuminata* could not grow positively as well as strictly heterotrophically, even if food is plentiful. Our results suggest that the main importance of phagotrophy in *D. acuminata* is to acquire a growth factor essential to photosynthetic growth besides obtaining the plastids from prey and that both light and prey are prerequisite for the growth and survival of *D. acuminata* (i.e. an obligate mixotroph).

108**COMPARISON OF GREEN ALGAL BLOOM INTENSITY AND RELATED WATER QUALITY PARAMETERS AT PAIRED “BLOOM” AND “NON-BLOOM” SITES**

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Green macroalgal blooms can create noxious odors and damage valuable marine ecosystems. This study examines green macroalgal cover over time as well as spatial distribution and oceanographic data for three pairs of inshore sites located in inland waterways of northern Washington State over the summer of 2006. The pairs were initially chosen to compare nearby high biomass and low biomass sites. Percent cover was measured along 50 m transect lines running perpendicular to the shoreline using randomly-placed 1 m² quadrats. Oceanographic data were gathered with data sondes anchored at -2 to -3 m MLLW. Water column and algal tissue nutrient concentrations were also determined. Sites in general showed trends toward increasing algal cover from June to August. The sites also showed greater than expected variability in salinity and temperature due to water depth and current.

109**UNDERWATER VIDEO ANALYSIS ALLOWS FOR THE MAPPING OF GREEN ALGAL BLOOMS THROUGHOUT THE INLAND MARINE WATERS OF WASHINGTON STATE**

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Ulvoid macroalgal blooms, or green tides, have a significant negative impact on shallow water habitats. The rapid growth of ulvoid algae has smothering and shading effects on eelgrass meadows, and some *Ulva* and *Ulvaria* species can release toxins into their environment. The invasion of eelgrass meadows by these blooms threatens the health of the eelgrass meadows themselves, as well as the health of organisms such as polychaetes, bivalves, and fish that live and breed among the eelgrass shoots. This study examines the presence of either the eelgrass *Zostera marina*, large-bladed green algae (Ulvaceae), or both at multiple sites in Puget Sound and neighboring waterways of Washington State. Underwater videography was conducted by the Washington State Department of Natural Resources and analyzed for the presence of seagrasses. We re-analyzed these videos for the presence of ulvoid algae at 88 sites for video taken in 2004. For 5 sites, we have also completed analysis for video taken in 2005. Substantial local variation was seen in the size and impact of green algal blooms. The five sites for which two years' data have been analyzed were found to have experienced some increase in ulvoid cover, with one site having experienced an exceptionally rapid and dramatic ulvoid algal bloom event. Both eelgrass and ulvoid monitoring using underwater videography is continuing, and this technique is proving to be useful in informing both the science of algal blooms and local policy decisions.

110**RECRUITMENT OF *PADINA AUSTRALIS* HAUCK (PHAEOPHYTA), AT SIRINART NATIONAL PARK AND TANG KHEN BAY, PHUKET PROVINCE, THAILAND**

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The brown algal genus, *Padina*, has a worldwide distribution in tropical and subtropical climate zones. It attaches to solid substrates and periodically may be partially or wholly buried in sand. *Padina* individuals are common and sometimes dominant in both the intertidal and shallow subtidal regions associated with coral reefs. We investigated, throughout one year, the recruitment of *P. australis* at Sirinart National Park (SNP) and Tang Khen Bay (TKB), Phuket Province. Experiments were carried out by clearing hard substrate 0.25 m² plots. At both SNP and TKB recruitment plots were established at 6 zones 20 m apart starting from the shore and out to 120 m, in order to assess the effects of tidal gradient on recruitment. Individuals were counted and percentage cover was calculated. The results showed that there was a significant difference in percentage cover by new individuals between the two locations ($P < 0.05$). However, during December 2005-June 2006, there

was no significant difference ($P > 0.05$). At SNP, the highest recruitment was found on the most upper zone, while at TKB recruitment occurred at all shore levels except at 80-100 m. The primary factor that influenced *P. australis* recruitment seems to be wave exposure. The SNP site is directly exposed to the open water which is especially high during the monsoon season, whereas TKB is a protected, sheltered site. **E-mails:** wbongkot@yahoo.com¹; larry.liddle@gmail.com²; anchana.p@psu.ac.th³

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INFLUENCE OF PHYSICAL TRAMPLING DISTURBANCE ON DESERT SOIL FOOD WEBS ASSOCIATED WITH BIOLOGICAL SOIL CRUSTS

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Biological soil crusts are a surficial community of cyanobacteria, lichens, algae, and mosses common to deserts around the world that stabilize soil surfaces and increase nutrient inputs to the soil food web through photosynthesis, biological nitrogen fixation, and mineral chelation. However, these crusts are susceptible to direct physical disturbance from trampling by humans and livestock. In a 10-yr field manipulation study we compared chemical and biological soil food web components (including protozoa, nematodes, and 16S t-RFLP microbial signatures) of chronically trampled plots to non-trampled control plots at three different sites in Canyonlands National Park, Moab, UT, varying in mineralogy and profile depth (10, 20 and 30 cm). We found that t-RFLP profiles of bacterial communities from trampled plots resemble non-crust soil more than well-developed crusts without trampling, a pattern that reflects a reverse development of late-successional stage crusts to early-successional stage crusts. Similarly, nematodes were generally more diverse in non-trampled plots than trampled plots and the microbivorous nematodes shifted to a greater proportion of bacterivores than fungivores and algivores in the trampled plots than the non-trampled plots. We also found idiosyncratic responses to trampling from several soil chemical and biological variables that were unique to a particular soil profile. For example, non-trampled plots had greater P, NO₃, and NH₄ concentration than trampled plots for the medium-depth profile but not the shallow or deep profile. Similarly, ciliates were more abundant in non-trampled plots than trampled plots only in the shallow- and medium-depth profiles while amoebae were more abundant in non-trampled plots than trampled plots only in the shallow-depth profile. This experiment supports the growing empirical evidence that physical disturbance negatively influences the function and composition of desert biological soil crusts and their associated food webs. However, our data also illustrates that the exact ecological changes that occur may depend on soil profile characteristics.

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MOLECULAR METHODS FOR INVESTIGATING NATURAL DIATOM COMMUNITIES: DELAWARE INLAND BAYS AND BEYOND

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Molecular techniques have revolutionized the way we examine microbial environmental samples. In the past, we have developed quantitative polymerase chain reaction (QPCR) primers and probes to enumerate specific phytoplankton species in an effort to understand the ecology of harmful algal species. Our current objectives were to design primers for a broad range of diatom species and species-specific QPCR probes for harmful diatoms, including *Pseudo-nitzschia australis*, *P. pungens*, *P. f. multiseriis*, *Chaetoceros convolutus*, and *C. concavicornis*. Diatom primers were constructed by aligning the 18S rRNA sequences from 92 full-length 18S rRNA sequences for diatoms found in Genbank. We developed primers for use in both denatured gradient gel electrophoresis (DGGE) and quantitative polymerase chain reaction (QPCR). We investigated the specificity of diatom-specific primers by sequencing clonal libraries of diatom species collected from several Delaware Inland Bays sites during the summer of 2006. Restriction fragment length polymorphisms were used to select clones for sequencing, and resulted in forty-eight unique banding patterns from two different primer sets. DGGE banding patterns were then compared to clone libraries to evaluate diatom diversity. In addition, QPCR probes are currently being developed to use in concert with the diatom specific primers to quantify harmful

algal species in the environment. In the future this research may be applied to identify and enumerate harmful diatom species in other parts of the world, as well as help researchers understand diatom community within the algal community.

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CYANOBACTERIA IN EUTROPHIC, TURBID IMPOUNDMENTS OF THE NORTH CAROLINA PIEDMONT

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We compared watershed land use, watershed area, reservoir morphometry (depth, surface area, volume), suspended solids (SS) and nutrient concentrations (total nitrogen [TN], total Kjeldahl nitrogen [TKN], nitrate + nitrite [NO₃⁻ + NO₂⁻, hereafter as NO₃⁻], total phosphorus [TP]), phytoplankton chlorophyll *a* (chl_a) concentrations, cyanobacteria assemblages, and microcystin concentrations from monthly data during summers of 2002-2006 in 29 potable water supply reservoirs (19-85 years old based on year filled) within the North Carolina Piedmont. The reservoirs were meso-/eutrophic and turbid (means > 25 µg TP/L, > 410 µg TN/L, > 6 mg SS/L). Under drought conditions there was a positive relationship between chl_a and both TN and TP, supported by correlation analyses and hierarchical ANOVA models. The models also indicated significant positive relationships between TN and TP, and between SS and both TP and TN. Agricultural land use was positively correlated with TKN for the reservoirs considered collectively, and with TN, TKN, TP, and chl_a in mod. reservoirs. In models considering reservoir age as a linear predictor, TN:TP ratios were significantly lower and NO₃⁻ was significantly higher in older reservoirs. Cyanobacteria assemblages comprised 60-95% of the total phytoplankton cell number. Potentially toxic taxa were dominated by *Cylindrospermopsis raciborskii*, *C. philippinensis* and *Plectonema wollei*, and numerous other potentially toxic taxa were common. Although known microcystin producers were low in abundance, microcystin (typically < 0.8 µg/L) was detected in most samples, and was as high as 27 µg/L. TP and chl_a were significant predictors of total cyanobacterial abundance. The data suggest that at present these turbid, meso-/eutrophic reservoirs have moderate cyanobacteria abundance and low microcystin levels over the summer growing season, even in low-precipitation seasons that favor cyanobacteria. Accelerated eutrophication from further watershed development is expected to promote increased cyanobacterial abundance including potentially toxic species.

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FIRST RECORDS OF PROTOSTELIDS (EUMYCETOZOA) FROM THE ANTARCTIC PENINSULA

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In December of 2004 and January of 2005 one of the researchers, Ina Timling, took part in an expedition to southern Argentina, the Shetland Islands, and the Antarctic Peninsula. During the expedition plant substrates were collected for laboratory culturing of slime molds and sent to the University of Arkansas Laboratory for Eumycetozoa Research. The collected plant substrates consisted of dead grasses and mosses. Sampling sites ranged from 54° 47' to 64° 53' south latitude. Workers at the eumycetozoa laboratory plated these substrates onto primary isolation plates in spring 2007. The majority of cultures showed no signs of protostelids, and only two species of protostelids were recovered, *Schizoplasmodiopsis vulgare* and a putative new species of the genus *Soliformovum*. These were identified on the primary plates by fruiting body morphology and morphology of amoebae. All protostelids recovered in the study were from the ground microhabitat with no protostelid species on aerial dead plant parts. Several isolates of both species were brought into single eukaryote culture with a known bacterial food source. The *Soliformovum* sp. strains that were moved into pure culture are not now fruiting, but were isolated from spores on *Soliformovum* sp. fruiting bodies on the primary plate, and the morphology of their amoebae is consistent with the genus *Soliformovum*. These are the first reports of protostelids from the Antarctic Peninsula, Palmer Archipelago, and Shetland Archipelago. These are also the first reports of protostelids with mosses on the ground as substrates.

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USING GUT CONTENT ANALYSIS TO ASSESS MACROALGAE IMPORTANCE AS A FOOD SOURCE FOR THE AMPHIPOD COMMUNITY ENDEMIC TO WESTERN ANTARCTIC PENINSULA

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Recent studies have revealed high abundances and diversities of crustacean mesograzers (especially amphipods) affiliated with benthic macroalgal communities along the Western Antarctic Peninsula. Reported densities have even been estimated as high as 50,000 individuals m⁻² algal tissue, illustrating the important ecological role amphipods may have in mediating mesograzer-algae interactions. Previous experiments have suggested that two amphipod species, *Gondogeneia antarctica* and *Proteobbingia gracilis*, significantly preferred feeding on the red alga *Palmaria decipiens*, while three species (*Desmarestia anceps*, *Desmarestia menziesii*, and *Plocamium cartilagineum*) were unpalatable in feeding assays. In contrast, amphipod density studies have revealed greater abundances of amphipods, including *G. antarctica* and *P. gracilis*, associated with the unpalatable species of algae. It is possible amphipods use unpalatable, and possibly chemically defended, macroalgae as a refuge from predation. Thus, although associations between amphipods and benthic macroalgae are clearly evident, the exact nature of these associations remains in question. Initial gut content analysis of a suite of amphipods collected on the Western Antarctic Peninsula was conducted and revealed a diverse array of prospective prey including diatoms, macroalgae filaments and thalli, bryozoans, sponge spicules, crustacean parts, and other non-diatom epiphytic unicellular algae. Initial results indicate most species have a mixed diet and many consume macroalgal filaments as a sizable portion of their diet, including some species thought to be strict carnivores. Although diatoms were found throughout the guts of many species, it is still unclear whether the presence of epiphytic material (i.e. diatoms) is the result of host material consumption and incidental ingestion of epiphytes or vice versa. It is important to discern key nutritional sources for these amphipods to further understand the processes mediating mesograzer-macroalgae interactions in near-coastal peninsular benthic habitats.

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THE ABILITY OF A SINGLE-CELL ALKALINE PHOSPHATASE ASSAY (ELF-97) TO INDICATE PHOSPHATE STRESS AND CELLULAR P CONTENT IN TWO STRAINS OF THE MIXOTROPHIC DINOFLAGELLATE, *PROROCENTRUM MINIMUM*

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Feeding in some mixotrophic dinoflagellates is influenced by nutrient concentrations, especially phosphate (P). To study the relationship between nutrient stress and mixotrophy in the field, it becomes necessary to have a reliable indicator of a cell's nutrient status. We tested a single-cell alkaline phosphatase assay (ELF 97) as an indicator of cellular P content and P stress in two strains of the mixotrophic dinoflagellate, *Prorocentrum minimum*. The strains were grown under nutrient-replete, P-, and N-limited conditions. Every other day for two weeks, cell numbers, alkaline phosphatase (AP) activity, and cellular P content were determined. The ELF assay worked well as an indicator of P stress and cellular P content in the first strain of *P. minimum*. This strain responded to P limitation by producing or activating AP (max. 67±7.5% of cells labeled), and its cellular P content was negatively correlated with AP activity. However, the second strain exhibited low AP activity throughout the experiment (max. 14±2.7% labeled), and cellular P content and AP activity showed no significant correlation. This was observed despite similar cellular P contents indicating a comparable degree of P stress in the two strains. The second strain may thus not be able to produce as much AP, and the ELF assay would fail to identify P stress and cellular P status. Assay results from the field would thus have to be interpreted with caution due to potential strain-specific differences.

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REGIONAL DISTRIBUTION OF NOTODENDRODID FORAMINIFERA IN MCMURDO SOUND, ANTARCTICA: IS *NOTODENDRODES ANTARCTIKOS* AN ENDANGERED PROTIST?

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Global biodiversity draws considerable geopolitical attention, but legislated actions for species conservation are biased toward higher taxa, particularly "darling" terrestrial taxa (e.g., mammals, birds), groups of commercial interest (e.g., timber, fishes) or unique terrestrial habitats (e.g., caves). Here we report on the regional distribution of a large (>1mm adult), distinctive protist, *Notodendrodes antarctikos*, which to date has only been found inhabiting a single cove in McMurdo Sound, Antarctica. As a highly endemic protist, *Notodendrodes antarctikos* serves as an excellent test case for the conservation of protistan diversity.

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CHEMICAL DEFENSES AGAINST DIATOM FOULING IN ANTARCTIC MACROALGAE: INSIGHTS FROM BIOASSAY GUIDED FRACTIONATION

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The prevention of fouling in marine algae involves complex processes that are not yet completely understood. Some antifoulant activity may be attributable to single compounds, whereas others may require synergistic interactions between collections of compounds. Antarctic marine benthic systems are a particularly relevant habitat for studying diatom antifoulants. This is because benthic diatoms are seasonally extraordinarily abundant. In a previous study, we investigated crude organic extracts of a broad suite of macroalgae from the western Antarctic Peninsula for toxicity against sympatric diatoms. In the present study, we bioassayed specific HPLC-generated fractions of crude extracts from two common brown macroalgae (*Georgiella confluens* & *Desmarestia antarctica*). Both species had shown significant activity against diatoms as crude organic extracts but not in feeding bioassays with sympatric herbivores. Diatom viability was measured with live/dead staining (fluorescein diacetate and Evans blue). Diatoms were exposed to macroalgal extracts at several concentrations (1x, 3x or 10x). Bioactivity was not detected at even 10 x in any of the 14 or 29 isolated fractions from *D. antarctica* and *G. confluens*, respectively. However, in both species a combination of all the isolated fractions at a 10 x concentration resulted in potent bioactivity with 99% diatom mortality. These findings indicate that in both species individual compounds are inactive in diatom antifoulant assays. Rather, some as yet unknown combination of these individual compounds results in potent biochemical synergy.

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PENTAPHARSODINIUM TYRRHENICUM IS A PARASITIC DINOFLAGELLATE OF THE CTENOPHORE *MNEMIOPSIS LEIDYI*

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Pentapharsodinium tyrrhenicum is a known benthic autotroph first described as genus *Peridinium* (Helogländer Meeres. 44:378) and reclassified in 1993 as genus *Pentapharsodinium* (J. Phycol. 29:223). While characterizing microbial assemblages of coastal *Mnemiopsis*, we identified a parasitic dinoflagellate previously described as a protoodiniid (Hydrobiologia 451:295) to actually be *P. tyrrhenicum*. *P. tyrrhenicum* is a common parasite of *Mnemiopsis* during the spring and summer months. It displays all stages of development (cyst, swimmer, and ectoparasite) within or upon the host, and it kills laboratory-held ctenophores. We routinely observe that coastal *Mnemiopsis* feed upon flocculant upper layers of shallow coastal sediments. Nearly all animals become intensely infested with *P. tyrrhenicum* cysts in the mesoglea immediately adjacent to the gut and food grooves. The highest concentration of ectoparasitic *P. tyrrhenicum* is on auricular and oral

lobes, where it ranges from $<1/\text{mm}^2$ to $>150/\text{mm}^2$. Although cysts and free-swimming cells are typically 30 μm in diameter, ectoparasites attached for >3 days are commonly irregular in shape and up to 200-300 μm in diameter. *P. tyrrhenicum* attaches to the epidermis with a peduncle, much like *Protoodinium* (Arch. Protistenk. 113:293). 18S rDNA analyses using 18S5F1/18SR1 primers (J. Phycol. 41:411) reveal 100% identity with *P. tyrrhenicum*. Calcofluor White staining (J. Phycol. 21:662) reveals the characteristic Po, X, 4', 3a, 7'', 4C +T, 4s, 5''', 2'''' plate tabulation of Balech. We conclude that *P. tyrrhenicum* is a parasite of southern *M. leidy*, and may play an important role in controlling ctenophore populations from the Chesapeake Bay to the Gulf of Mexico. We thank D. Coats, F. Chen and E. Wommack for cruise support. Supported by Alabama EPSCoR NSF EPS-0447675; The Center for Environmental & Cellular Signal Transduction; AU College of Science & Mathematics Cell / Molecular Biology; NSF MCB 0348327 to AGM, AR, RG & BS.

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MACROALGAL COLONIZATION: RECRUITMENT OF A NEW ROCKY HABITAT ALONG THE SOUTH TEXAS COAST

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Marine macroalgal assemblages on artificial structures may play an important ecological role in coastal and estuarine ecosystems. These communities may supplement natural communities in nearby waters. Variations in abundance and diversity of algae aid in the assessment of habitat function and ecosystem health. Macroalgae found on the rocky jetties of Port Aransas and Mansfield Pass have been previously characterized, but the jetties of Packery Channel, located in between, represent a recent habitat addition. The purpose of this research was to monitor the initial recruitment of macroalgal species during the first year of colonization. Eight sampling sites were established along the offshore portion of the new Packery Channel jetties. Samples were taken bimonthly from along a ten meter transect between September 2006 and July 2007. Quadrats (20 X 30cm) were sampled every meter by harvesting techniques and the use of an airlift. All plant materials were identified to lowest possible taxon and biomass calculated. Data obtained from this study assesses composition and establishes a timeline for algal recruitment to the pass. Within the first month after jetty completion, 20 species of macroalgae had become established, and at six months species richness had increased to 32. Preliminary Primer analyses show strong linkages between rate of recruitment and level of wave energy. Sites with the highest level of wave energy tended to have the highest overall biomass and species richness.

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PROTISTAN DISTRIBUTION IN RELATION TO SPATIAL VARIATIONS OF EXTREME GEOCHEMICAL PARAMETERS IN THE RIO TINTO, SPAIN

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The microbiology of the Rio Tinto, Spain is being studied intensively because it may be unique in age and scale among acidic aquatic sites with high concentrations of heavy metals. Most studies of systems with similar physico-chemical characteristics (pH below 3 and high concentrations of heavy metals) have been done in relatively localized natural systems such as volcanic fumarole areas or in acid mine drainage systems. A feature that stands out in the 90-km-long Rio Tinto is the community of photosynthetic protists that form conspicuous biofilms whose biomass often exceeds that of their chemolithotrophic counterparts. While the Rio Tinto has certainly been impacted by mining activities, recent paleontological evidence demonstrates that the acidic conditions have persisted for hundreds of thousands of years. This study explores the relationship between the physico-chemical environment and the protistan community at three stations along the river with distinct characteristics. Multivariate methods including Canonical Correspondence Analysis were used to analyze triplicate samples from three sites at each station for both geochemical and diversity data. This design permits us to explore patterns of protistan diversity at various scales with respect to environmental parameters.

DINOFLAGELLATE/CYANOBACTERIA CONSORTIA IN THE TROPICAL INDIAN OCEAN AND THE NORTH-WEST AUSTRALIAN SEA

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The horizontal and vertical distribution of dinoflagellates with ectosymbionts and of physico-chemical parameters were investigated along a transect from Cape Town, South Africa to Broome, Australia, on a cruise carried out in October-November 2006. We found 4 genera of dinoflagellates, which had ectosymbionts: *Ornithocercus*, *Histioneis*, *Parahistioneis* and *Citharistes*. The highest cell concentrations were found in the upper 100 m, indicating that the consortia are based on photosynthesis. However, some dinoflagellates with ectosymbionts were found as deep as 400 m. Cell concentrations of the consortia were positively correlated with water temperature. Otherwise, the cell concentrations of the consortia were negatively correlated to the NO₃⁻ concentration, indicating that the consortia are most successful in N-limited environments. The light and transmission electron microscopy of *O. magnificus* and *O. quadratus* revealed that ectosymbionts were cyanobacteria. In addition, bacteria were sometimes found on the girdle list and on the sulcal list of these dinoflagellates, where they may provide favorable condition for cyanobacterial N₂ fixation. Light microscopy of *O. magnificus* and *O. quadratus* cells revealed that the dinoflagellates ingested the ectosymbionts. Transmission electron microscopy further documented ingested material inside the dinoflagellates. However, the origin of the ingested material could not be determined and thus it cannot be ruled out that the dinoflagellates ingested other food items.

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CHARACTERIZATION OF THE ENDOSYMBIOTIC DINOFLAGELLATES FROM SORITICEAN FORAMINIFERA

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We found no obvious plates on the surface of any of our isolates even though we made a number of different attempts to find them. We also failed to find plates in a detailed TEM study of symbionts in hospitae in *Marginopora vertebralis* from Heron Island. Plastids are very different from those described by Freudenthal (1962) in his diagnosis of *Symbiodinium*. The plastids of our study group fell into 2 groups: 1) those that were petal-like (GU, LI, AH, AS, HA, RB, and ZA) and those that were net-like or reticulate (TA, BI, CA and CF). Considerable morphological differences in plastid structure among the isolates were recognized. The incredibly skilled quantitative transmission electron microscopical study of Blank (1987) of *Symbiodinium* sp. from *Montipora verrucosa* was certainly an elegant approach to image, in 3-D reconstructions from serial sections, the tiny chromosomes of those symbionts they studied. However, methodologies based upon confocal microscopical imaging are now as accurate, and perhaps more practical, approaches to the same data. We have developed a method to get DAPI stained chromosome spreads in the confocal microscope and by using proprietary programs to measure and calculate the sizes and volumes of nuclei in optical slices of maximum diameter relative to rest of the cell in the slice we have found differences in the isolates in our study group. The ratio of the diameter of the nucleus to the diameter of the cell varied greatly; CA and ZA had nuclei slightly over 1/3 the diameter of the cell while most of the other isolates, TA, RB, HA, GU, AS, and AH have smaller nuclei that occupy ~20-25% of their cells. Isolates differ in life cycle patterns. Three of our study group Cas-F, Cas-A and TA had some motile forms all day long and there were other isolates in which we never observed motility. With the unaided eye one could discern life cycle-behavioral differences through the test tube walls. Some isolates (AH, AS) form rings on the walls of the tubes at the air-water interface of test tube cultures and other isolates (CA, CF) form vertical streaks on the side of tubes facing away from the light source. (Supported by PSC-CUNY grant 64234-00 35).

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ULTRASTRUCTURAL CHARACTERIZATION OF THE LYTIC CYCLE OF AN INTRA-NUCLEAR VIRUS OF *CHAETOCEROS WIGHAMII* FROM CHESAPEAKE BAY, USA

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Marine viruses are widely distributed in the ocean where they infect organisms ranging in size from bacteria to whales. Numerous microalgal species are infected by viruses that have the potential of control phytoplankton dynamics by reducing host populations, preventing bloom formation, or causing the collapse of blooms. Here we describe a virus infecting the diatom *Chaetoceros wighamii* from the Chesapeake Bay. To characterize the morphology and lytic cycle of this virus, we conducted a time-course experiment, sampling every 4 hours over 72 hours following viral inoculation. In vivo fluorescence began to decline 16 hours after inoculation and was reduced to less than 19% of control cultures by the end of experiment. Transmission electron microscopy (TEM) confirmed infection within the first 8 hours of inoculation, as indicated by the presence of virus-like particles (VLP) in the nuclei (range 2-9% prevalence). In addition, the percentage of cells with VLP showed two peaks, at approx. 20 and 44 hours of inoculation, suggesting two cycles of VLP production. VLPs were present in two different arrangements: rod-like structures that appeared in cross-section as paracrystalline arrays of hexagonal-shaped profiles measuring 12 ± 2 nm in diameter; and uniformly electron-dense hexagonal-shaped particles that lacked a tailed and measured approximately 22-28 nm in diameter. Nuclei containing paracrystalline arrays were most prevalent early in the infection cycle, while cells containing VLPs increasing and then declining towards the end of the cycle. The proportion of nuclei containing both paracrystalline arrays and VLPs remained relatively constant. This pattern suggest that rod-like, paracrystalline arrays fragmented to produce icosahedral VLPs. Potential burst size of CwNIV based on analysis of TEM images averaged 26,400 viruses per infected cell, considerably higher than previously estimated for diatom viruses, but similar to the burst size of *Heterosigma akashiwo* viruses.

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HIGH CO₂, UV, AND CARBON PARTITIONING IN *THALASSIOSIRA PSEUDONANA*

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Although several different environmental factors (inc. CO₂ availability, temperature, ocean water pH, UV radiation) are subject to global climate change, rarely has the combined influence of these factors on biological processes been investigated. Previous studies indicate that exposure to UV radiation can reduce carbon assimilation and alter the pattern of fixed carbon allocation in both cultured and natural populations of phytoplankton, such that the synthesis of nitrogen-rich compounds is conserved under ecologically relevant UV-B conditions. These changes have implications not only for the cells in questions, but also for higher trophic levels. Here, allocation of fixed carbon to carbohydrates, proteins, and lipids in *Thalassiosira pseudonana* (3H) during exposure to UV radiation is examined at conditions mimicking current and high atmospheric CO₂. Cultures grown at 200 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ in the absence of UV and either 380 or 1000 ppmv CO₂ were subjected to short-term exposures to saturating irradiance with a polychromatic spectrum. Subtle differences in allocation patterns were observed between the different CO₂ conditions at both UVA and UVB regions of the spectrum. These differences will be examined through the use of biological weighting functions for the UV response.

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INTRA- AND INTERSPECIES DIFFERENCES IN *PSEUDO-NITZSCHIA* ECOPHYSIOLOGY, GENETICS AND TOXICITY

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Clonal cultures of plankton are widely used and have contributed greatly to knowledge of microbial systems. However, many physiological characteristics vary drastically between strains of the same species, calling into

question our ability to make ecologically relevant inferences about populations based on studying one or a few strains. In this study, I used sixteen strains of three species of the diatom *Pseudo-nitzschia* isolated primarily from the mid-Atlantic coastal region of the United States. Toxin (domoic acid) production and growth rates were measured in cultures using different nitrogen sources (NH₄, NO₃ and urea) and growth irradiances. Strains were positively identified using molecular (ITS and LSU) techniques and morphological characteristics. The strains exhibited broad differences in growth rate and toxin content, even between strains of the same species isolated from the same water sample. Both *P. multiseriis* clones produced toxin, yet utilize nitrogen sources differently. Only some of the *P. calliantha* and *P. fraudulenta* isolates were toxic and domoic acid content varied by orders of magnitude. All species had variable intraspecies growth rates on each nitrogen source, but *P. fraudulenta* strains had the broadest range. Growth versus irradiance curves show temperature and species effects on light-limited growth and maximum growth rate. These findings show the importance of defining intra- and interspecies variability in ecophysiology and toxicity. Ecologically relevant functional diversity in the form of ecotypes or cryptic species appears to be present in the genus *Pseudo-nitzschia*.

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TEMPERATURE AND IRRADIANCE IMPACTS ON THE GROWTH, PIGMENTATION AND PHOTOSYSTEM II QUANTUM YIELDS OF *HAEMATOCOCCUS PLUVIALIS* (CHLOROPHYCEAE)

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The microalga *Haematococcus pluvialis* Flotow has been the subject of a number of studies concerned with maximizing astaxanthin production for use in animal feeds and for human consumption. Several of these studies have specifically attempted to ascertain the optimal temperature and irradiance combination for maximizing growth rates of *H. pluvialis*, but there has been a great deal of disagreement between laboratories. “Ideal” levels of temperature and irradiance have been reported to range from 14 to 28 °C and 30 to 200 μmol m⁻² s⁻¹. The objective of the present study was to simultaneously explore temperature and irradiance effects across an experimental region that encompassed all of the reported “optimal” combinations of these factors. To this end, a two-dimensional experimental design based on response surface methodology (RSM) was created. This approach allowed us to explore temperature-irradiance interactions within a comprehensive and statistically valid framework. Maximum growth rates were achieved at 27 °C and 260 μmol m⁻² s⁻¹, while maximum quantum yield of stable charge separation at photosystem II (Fv/Fm) was achieved at 27 °C and 80 μmol m⁻² s⁻¹. Maximum pigment concentrations correlated closely with maximum Fv/Fm values. As expected, no significant astaxanthin production was found across the design space. Numeric optimization of growth rate and quantum yields produced an optimal combination of 27 °C and 250 μmol m⁻² s⁻¹. Polynomial models of the various response surfaces were validated with multiple points and were found to be very useful for predicting several *H. pluvialis* responses across the entire two-dimensional design space.

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AN IMPROVED METHOD FOR PHYCOBILIN EXTRACTION

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Remote sensing methods have a goal of estimating plant populations accurately. With many plants, particularly in the green plant lineage, chlorophyll is the most abundant pigment. In cyanobacteria, however, the phycobilin pigments predominate. A number of methods have been used to quantify phycobilins from cyanobacteria. We investigated use of asolectin-CHAPS buffer to remove these pigments and compared the removal efficiency to the traditional phosphate buffer extraction method. For several species of cyanobacteria, 30% higher concentrations of phycocyanin were removed using the new method. An estimation of removal efficiency was made using residual fluorescence in the cell pellet-the asolectin-CHAPS method removed over 75% of the pigment, whereas the phosphate buffer method removed less than 30%. Field samples were assessed for the co-relationship with remotely sensed pigments-in all cases the asolectin-CHAPS method provided better model fit.

COMPARATIVE PHYSIOLOGY OF DESERT BIOTIC CRUST ALGAE (CHLOROPHYTA) AND THEIR AQUATIC RELATIVES: EXPLORING DESICCATION TOLERANCE AND PHOTOPROTECTION

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Recent molecular data indicate that desert green algae have evolved from freshwater ancestors at least 14 times in Chlorophyta. These multiple independent origins offer a unique opportunity to study the adaptation of photosynthetic organisms to life on land, in a comparative phylogenetic framework. We investigated the photorecovery of phylogenetically matched desert and aquatic algae following desiccation in darkness and under illumination. Isolates of six different desert algae survived extended periods of desiccation (at least 4 weeks) when dried in darkness, and rapidly recovered high levels of photosynthetic quantum yield when rehydrated in the dark. However, when 4 weeks of desiccation was accompanied by illumination, half of the desert taxa lost their ability to recover quantum yield during rehydration in the dark. Aquatic algae, in contrast, recovered very little during dark rehydration following even just 24 hours of desiccation. Re-illuminating algae after they had been rehydrated produced a nearly complete recovery of quantum yield in all desert and two of five aquatic taxa. These contrasts provide physiological evidence that desert green algae possess means of desiccation tolerance and photoprotection that are distinct from those in aquatic relatives, corroborating molecular evidence that they are not happenstance, short-term visitors from aquatic environments. Examining the induction of non-photochemical quenching (NPQ) in two pairs of fully hydrated, dark-adapted desert and aquatic taxa at a range of light intensities from dark to 800 μE revealed marked differences in the development of NPQ at high light. Desert algae developed lower levels of NPQ than aquatic relatives at high light intensity, possibly at least in part because screening pigments protected the photochemical apparatus in desert taxa at high light.

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PHOTOCHEMICAL PRODUCTION OF H_2O_2 IN BUFFERED MEDIA REDUCES *PROCHLOROCOCCUS* GROWTH

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Zwitterionic “Good’s” buffers (e.g. HEPES, TAPS) have been shown to form hydrogen peroxide (H_2O_2) when exposed to light, potentially making them poor choices in media used for culturing phototrophic microorganisms. We tested natural seawater-based media containing seven such buffers, as well as the singly ionizable buffer Tris, by incubating them either under light (sterile or inoculated with the cyanobacterium *Prochlorococcus*) or in the dark (sterile only). H_2O_2 levels of sterile Good’s buffer samples changed little in dark-incubated samples but rose dramatically in samples exposed to light. Conversely, H_2O_2 accumulation was minimal in Tris or unbuffered samples. *Prochlorococcus* growth rates were significantly ($p < 0.05$) lower in samples whose sterile counterparts exhibited high H_2O_2 maxima ($> 1.5 \mu\text{M}$). Since H_2O_2 formation does not appear to degrade the buffer molecules, we propose that Good’s buffers catalyze H_2O_2 formation through a mechanism involving a nitrogen-centered radical. Recommendations for using buffers to culture phytoplankton are discussed.

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TRADITIONAL VS. INTEGRATED AQUACULTURE OF *GRACILARIA CHILENSIS* C.J. BIRD, MCLACHLAN & E.C. OLIVEIRA: PRODUCTIVITY AND PHYSIOLOGICAL PERFORMANCE

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Species within the genus *Gracilaria* are known as one of the main sources of agar. Furthermore, several studies recommend species of *Gracilaria* as efficient biofilters in Integrated Multi-Trophic Aquaculture

(IMTA) systems designed to mitigate the environmental problems caused by finfish or other forms of fed aquaculture. *Gracilaria chilensis* is commercially cultivated in Chile and this industry continues to expand. One of the problems faced by the traditional *Gracilaria* farms is the drop of production related to seasonal changes in N availability and irradiance. IMTA may offer a solution to some of these problems as they will provide a continuous stream of nutrients for *Gracilaria* culture and sustain satisfactory growth rates. In this study, performed at southern Chile, cultivation systems were set close to a salmon farm and at two traditional *G. chilensis* cultivation areas that were away from the influence of salmonid aquaculture. Data were collected during the austral summer. Since nutrient availability can effect algal photosynthetic responses, data on Fv/Fm, Φ PSII, together with tissue contents (C, N) were measured to evaluate the physiological performance of *G. chilensis* in each treatment. Simultaneously, growth rates were also measured and productivity in each treatment was determined. Advantages of establishing *Gracilaria* commercial cultures in IMTA systems are discussed.

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TYROSINE PHOSPHORYLATION OF CILIARY BASAL BODIES IN *TETRAHYMENA THERMOPHILA*

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We have found that a monoclonal antibody to phospho-tyrosine (p-tyr-100, Cell Signaling) strongly and preferentially labels somatic and oral ciliary basal bodies of *T. thermophila*, but only weakly labels cilia. Ciliary basal bodies are modified centrioles that serve many important functions, particularly in relation to development and activity of cilia. We have therefore begun to analyze the functional significance of the labeling we have observed. Presently, we are attempting to establish that the labeling patterns are due to activity of enzymes responsible for phosphorylation and dephosphorylation of tyrosine, by testing effects of enzyme inhibitors on labeling by P-tyr-100. So far, we have tested Tyrphostin 25, an inhibitor of receptor tyrosine kinases (RTKs; e.g. epidermal growth factor receptor kinase), and as a control, LY294002, an inhibitor of PI3-kinase that should not directly phosphorylate tyrosine. Stock solutions of LY294002 and Tyrphostin 25 were prepared in DMSO. Late exponential phase cells (*T. thermophila* strain Cu427.4) were washed with tris buffer, treated for 30 min at RT with drug diluted in DMSO or DMSO alone as control, fixed for 30 min with buffered formalin, mounted on slides and examined. LY294002 was tested at 10 μ m, which is sufficient to completely inhibit nascent phagosome closure during phagocytosis in *T. thermophila* (Zackroff, Octavio and Hufnagel, unpublished). Tyrphostin was tested at concentrations ranging from 0.5 to 1.5 μ M. Cells treated with LY294002 showed no change in labeling of ciliary basal bodies by the P-tyr-100 antibody compared with the DMSO control. Cells treated with Tyrphostin 25 also did not show significant affects on labeling of basal bodies. Cells treated for longer periods (3 hr, 6 hr and overnight) with 0.5 μ M Tyrphostin in 0.1% DMSO did not exhibit significant changes in basal body labeling with time. To determine whether Tyrphostin 25 inhibits processes in *Tetrahymena*, known to be due to receptor tyrosine kinases, further experiments are planned. If this is confirmed, we will continue to look for a non-receptor tyrosine kinase-mediated process to explain the phospho-tyrosine labeling of basal bodies in *Tetrahymena*.

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GpMyoF, A WD40 REPEAT-CONTAINING MYOSIN ASSOCIATED WITH THE MYONEMES OF *GREGARINA POLYMORPHA*.

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This work presents the first characterization of a WD40 repeat-containing myosin identified in the apicomplexan parasite, *Gregarina polymorpha*. This 222.7 kDa myosin, GpMyoF, contains a canonical myosin motor domain, a neck domain with six IQ motifs, and a tail domain containing regions of predicted coiled-coil structure and most notably, multiple WD40 repeats at the C-terminus. In other proteins, such repeats assemble into a β -propeller structure implicated in mediating protein-protein interactions. Immunolocalization studies suggest that GpMyoF is localized to the actin-rich annular myonemes that gird the parasite cortex. Extraction studies indicate that this myosin shows an unusually tight association with the cytoskeletal fraction and can only be solubilized by treatment with high pH (11.5) or the anionic detergent sarkosyl. This novel myosin and its homologs, which have been identified in several related genera, appear to be unique to the Apicomplexa and represent the only myosins known to contain the WD40 domain. The

function of this myosin in *G. polymorpha* or any of the other apicomplexan parasites remains uncertain but the protein is poised to play roles not just in reinforcing cellular architecture but also in driving the bending or peristaltic motions exhibited by some gregarines.

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CELLULOSE SYNTHASE (*CesA*) GENES IN THE RED ALGA *PORPHYRA YEZOENSIS* UEDA

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As in land plants, the cellulose microfibrils of the conchocelis phase of red algae from the genus *Porphyra* are synthesized by clusters of cellulose synthase enzymes ("terminal complexes"). However, the morphologies of *Porphyra* terminal complexes and the cellulose microfibrils they produce differ from those of land plants (J. Phycol. 30:300). In an effort to characterize the genetic basis for these differences, we have identified a cellulose synthase (*CesA*) gene from *Porphyra yezoensis* Ueda strain TU-1. Based on similarity to a *CesA* from the moss *Physcomitrella patens*, a single sequence was identified in a database of 20,779 *P. yezoensis* EST sequences (DNA Res. 7:223). The conceptual translation of the corresponding 1093 bp cDNA clone (provided the Kazusa DNA Research Institute) includes two of the four catalytic domains that characterize *CesA* proteins. Using TAIL PCR with primers based on the cDNA sequence, we amplified four *CesA* fragments from *P. yezoensis* TU-1 genomic DNA. Primers based on the contig assembled from these sequences were then used to amplify a nearly full-length genomic *CesA* sequence that hypothetically encodes all four *CesA* catalytic domains and the expected N- and C-terminal transmembrane domains. When this sequence was used for a second BLAST search of the *P. yezoensis* EST database, four additional ESTs with nearly perfect identity to the query sequence were identified. One EST appears to represent a full-length cDNA clone. These EST sequences were not identified in the original search because of their substantial differences from land plant *CesAs*. The *P. yezoensis CesA* sequence is most similar to cyanobacterial *CesA* genes and a class of *CesA-like* genes present in *Physcomitrella patens* and *Selaginella moellendorffii*, but not spermatophytes. We are currently preparing Southern blots to estimate the number *CesA* genes in the *P. yezoensis* genome. Comparing the *CesA* genes of red algae and land plants may lead to the identification of sequences that control terminal complex and cellulose microfibril morphology. This research is supported by a grant from the Rhode Island Science and Technology Advisory Council.

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MOLECULAR CLONING AND GENE EXPRESSION ANALYSIS OF NITRATE REDUCTASE FROM THE HARMFUL MARINE ALGA, *HETEROSIGMA AKASHIWO*

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Heterosigma akashiwo and *Chattonella subsalsa* are harmful raphidophyte species that form mixed blooms in Delaware's Inland Bays. Previous work demonstrated that *H. akashiwo* has a distinct preference for nitrate while *C. subsalsa* grows to higher density on ammonium as a nitrogen source. In an effort to understand the ecology of these algae, the molecular regulation of nitrogen assimilation was investigated for *H. akashiwo*. The reduction of nitrate to nitrite is catalyzed by nitrate reductase (NR) and is often considered to be the rate-limiting step in nitrate assimilation. Although NR has been studied extensively in vascular plants and a few species of green algae and marine diatoms, little is known about induction and regulation of NR gene expression in other phytoplankton species. Here, we describe the complete NR gene sequence and investigate the regulation of NR gene expression in *Heterosigma akashiwo*. NR mRNA was measured using quantitative real-time PCR after a pulse of nitrate was supplied to cultures that were N-starved and N-replete, as well as cultures supplied with ammonium as the sole nitrogen source. The results demonstrate that NR is constitutively expressed in *H. akashiwo*, even in the absence of nitrogen. In addition, the rate of increase in NR mRNA after spiking nitrate into N-starved cultures is greater than any other algal species tested to date. NR is also expressed in cultures grown with ammonium as a nitrogen source, suggesting that ammonium does not inhibit NR expression in this species. Addition of nitrate to N-replete cultures, however, resulted in a decrease in NR expression, and implies that products of nitrogen assimilation, such as glutamine, may act to repress NR expression in *H. akashiwo*. These results, along with NR enzyme activity data (presented elsewhere), suggest that *H. akashiwo* and *C. subsalsa* do not compete for the same nitrogen source, and provide a possible

explanation for mixed blooms of these species in Delaware's Inland Bays.

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BIOCHEMICAL CHARACTERIZATION OF *ENTAMOEBEA HISTOLYTICA* ALCOHOL DEHYDROGENASE 2 (EhADH2) AND A NOVEL *E. INVADENS* BIFUNCTIONAL ALCOHOL DEHYDROGENASE (EiADHE)

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The ADHE family of bifunctional acetaldehyde dehydrogenase (ALDH)/alcohol dehydrogenase (ADH) enzymes appears to have arisen from the fusion of independent genes that encode the ALDH and ADH enzymes. ADHE enzymes have been reported in firmicutes, proteobacteria, cyanobacteria, and a few eukaryotes (*E. histolytica*, *Giardia intestinalis*, *Mastigamoeba balamuthi*, chlorophyte algae, and *Piromyces* sp. 2). Here we report the cloning, expression and initial characterization of a new member of the ADHE family in *Entamoeba invadens*, a reptilian pathogen. The nucleotide sequence is 2642 base pairs long and encodes an 880 amino acid polypeptide. The amino acid sequence is 86% homologous to the *Entamoeba histolytica* EhADH2 protein. The EiADHE sequence suggests the presence of two conserved domains: alcohol dehydrogenase (ADH) and acetaldehyde dehydrogenase (ALDH), as well as the iron-binding domain and a catalytic histidine. We have expressed the EiADHE gene in a mutant strain of *E. coli* carrying a deletion of the *adhE* gene. Expression of EiADHE restored the ability of the mutant *E. coli* strain to grow under anaerobic conditions. Preliminary analyses show ADH and ALDH activities in vitro. Additional molecular and biochemical analyses are in progress. Inhibition studies will have clinical implications for amebiasis in humans and reptiles.

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MOLECULAR MECHANISMS OF IRON ACQUISITION BY DIATOMS

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It is well known that diatoms require iron for photosynthesis and other metabolic processes; however, the mechanisms different diatoms use to acquire iron are poorly understood. *Pseudo-nitzschia* spp., diatoms commonly found to bloom from iron addition experiments in iron-limited high nitrate, low chlorophyll ocean waters, have been shown in laboratory studies to acclimate to iron-limitation via a copper-dependent mechanism. In contrast, other diatoms, such as *T. pseudonana*, can be readily starved for iron in the laboratory. We are interested in elucidating the variations in iron uptake strategies utilized by different diatom species. Our investigation begins with isolating genes in *T. pseudonana* with homology to components of copper-dependent, high affinity iron uptake systems described in fungi and fresh water algae (e.g. ferric reductases, copper-dependent ATPases and multi-copper oxidases). The potential iron-status markers, ferredoxin and flavodoxin, are also being targeted for analysis. We are quantitatively assaying expression patterns of these genes under iron and copper replete and limiting conditions. Differences in the genetic induction of these systems should help explain disparate physiological responses to iron stress in individual diatom species and may explain why certain species persist in different oceanic regions.

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ENCYSTMENT OF *ACANTHAMOEBA CASTELLANII* INVOLVES STORAGE PROTEINS IN THE FORM OF FRAGMENTED ACTINS AND CUPIN DOMAIN CONTAINING PROTEINS AS DETECTED BY TWO-DIMENSIONAL GEL ELECTROPHORESIS AND MASS SPECTROMETRY

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The life cycle of *A. castellanii* consists of two stages, the trophozoite and the cyst. To investigate the factors mediating encystations, we performed global proteomic analysis of the *A. castellanii* by MALDI-TOF-MASS and blast search. More than 1000 protein spots were visualized on BCB stained 2-D gels with high resolution and reproducibility and identified more than 200 proteins. During the encystation, the most interesting change

was degradation of an actin-1 and accumulation of fragmented actins. Compared with only one actin in trophozoite, we detected 8 actin fragments, 4 actin-related proteins and one actophorin in cyst. The level of actin-1 (pI 5.86, 41.1kDa) detected in the trophozoite was decreased with encystment and disappeared in 6-h cysts. The level of an actin-1 fragment (aa 148-336; pI 5.84, 24.6 kDa) was high in mature cysts. The level of 4 actin-related proteins (pI 7.03, 58.8kDa; pI 5.94, 58.2kDa; pI 7.32, 57.2kDa and pI 7.19, 28.2kDa) increased with encystment and maintained constant level during the cyst maturation. The level of actophorin (pI 6.25, 14.9kDa) was continually increased in all stages of differentiation. In a search of conserved domains, we identified 2 proteins containing cupin domains (pI 6.15, 24.3 kDa and pI 4.84, 26.8 kDa). Cupin domains are well conserved among seed storage proteins, phaseolin in *Phaseolus vulgaris* (kidney bean) and glutelin type-B 1 precursor in *Oryza sativa* (rice). The levels of HSP 70 (pI 6.52, 41.7kDa) and BAH domain containing proteins (pI 6.30, MW 27.0kDa) homologous to MTA1 (metastasis-associated protein) were also increased in the process of the encystment. Thus, the encystment process may involve with accumulation of amino acids in fragmented actins and cupin domain containing proteins associated with HSP 70 and signal transduction associated with MTA1

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INACTIVATION OF *GIARDIA* STRAINS: COMPARISON OF TEST METHODS

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Giardia lamblia (syn *intestinalis*, *duodenalis*) is one of the most important enteric parasites in the world; species infect a wide range of organisms including birds, and mammals. Giardiasis is the most common gastrointestinal disease of protistan etiology. Development of disinfection methods is currently aimed at inactivation of the parasite in water supplies using ultraviolet irradiation and on fruits and vegetables using gamma-irradiation due to the low cost and lack of harmful residue. The "gold standard" used to measure the inactivation dose for *Giardia* cysts is the Mongolian gerbil infectivity assay. A more sensitive means of assessing inactivation was achieved by determining inactivation doses of trophozoites. Since trophozoites are more resistant to radiation than cysts, the dose required for the same inactivation level of trophozoites is ten-fold higher than that of cysts. In addition, it is possible to collect data from different strains of *Giardia* much more quickly, and with a significant decrease in cost. Five strains of *Giardia lamblia* (WB, WB-C6, P-1, D3, and GS) were tested for inactivation by both methyl methanesulfonate (MMS) and gamma-radiation. Two methods were used to determine the level of inactivation, a modification of the Reed and Meunch technique in 96-well plates, and counts of motile trophozoites in tubes of media with a hemacytometer. From the data collected, curves that compare percent control versus dose were within the standard error measurements of the two treatments indicating that the data generated were very similar. Data from the five different strains of *Giardia* indicated that the inactivation level was similar for all tested strains. Since both Assemblages A and B were tested (i.e., the only two Assemblages isolated from humans), the doses of gamma-radiation required for inactivation should be adequate for control of the spread of giardiasis in humans.

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THE USE OF cDNA LIBRARIES TO INVESTIGATE ASEXUAL REPRODUCTION IN *PORPHYRA UMBILICALIS* (L.) KÜTZING

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We are investigating gene expression during asexual differentiation in *Porphyra umbilicalis* (L.) Kützing. This red alga is a suitable model organism for studying differences in gene expression during reproduction. *Porphyra umbilicalis* can reproduce sexually in the northeastern Atlantic, but a metapopulation of this alga appears to have lost this ability in at least part of the northwestern Atlantic (The Gulf of Maine). We constructed λ phage cDNA libraries from poly (A)⁺ RNA extracted from vegetative, asexually differentiating cells, and mature neutral spores of *P. umbilicalis* from the Maine (USA) shore. The initial analysis of 90 recombinant clones from the differentiating library yielded 44 for which the insert sequences were similar to those found in other *Porphyra* EST libraries. The polypeptides encoded by 9 ESTs had significant similarity to polypeptides present in other *Porphyra* species, and many of the ESTs encoded proteins homologous to

polypeptides of other organisms that function in photosynthesis, regulatory processes, and metabolism. More than a third of the 90 characterized sequences lacked homology to sequences deposited in public databases. Further analyses of the libraries should help us identify the suite of genes that are active during asexual differentiation. The libraries created in this study will be useful in annotating sequences generated in the Joint Genome Institute's upcoming *Porphyra* genome project.

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THE SEARCH FOR HORIZONTAL GENE TRANSFER FROM A HETEROKONT ALGA TO A KLEPTOPLASTIC SEA SLUG

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Early in development the sacoglossan mollusc *Elysia chlorotica* feeds on and acquires chloroplasts from the heterokont alga *Vaucheria litorea*. This symbiotic (or kleptoplastic) association sustains the sea slug photoautotrophically for its entire ten month life-cycle in the absence of any additional algal food supply; only light and a source of CO₂ are required. To date, no algal nuclei have been detected within the cells of the mollusc and the 115 kb chloroplast genome codes for less than 10% of the essential chloroplast proteins needed to sustain photosynthetic activity. Horizontal gene transfer (HGT) of algal nuclear genes to the sea slug nuclear and/or mitochondrial genome(s) is a more plausible source for the essential nuclear-encoded chloroplast-targeted proteins. To determine if any algal genes have been transferred into the sea slug mitochondrial genome, we have sequenced the entire mitochondrial genome using multiple PCR reactions and primer walking. The genome is 14,573 bp and encodes 15 genes including: cytochrome oxidase subunits I, II and III, ribosomal RNAs (small and large subunits), cytochrome b, nad 1-6, nad 4L, and ATP synthase subunits 6 and 8. Twenty-four tRNAs were also identified. Detailed analysis of the mitochondrial genome to reveal any algal HGT events and phylogenetic comparisons will be presented.

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INACTIVATION OF EITHER ONE OR BOTH OF THE TWO GENES THAT ENCODE FIBRILLIN IN *SYNECHOCYSTIS* PCC6803 IMPAIRS GROWTH AND ALTERS CELL ULTRASTRUCTURE FOR CULTURES GROWN UNDER HIGH IRRADIANCE

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A diverse family of polypeptides referred to as fibrillins or plastid lipid-associated proteins are present in virtually all oxygenic photosynthetic organisms but in no other life forms. In land plants, fibrillins may be involved in protecting the photosynthetic apparatus from photooxidative damage. At least eleven different fibrillin genes, with considerable diversity in sequence, exist in higher plants. The situation is much simpler in cyanobacteria, which contain only one or two such genes. To gain an understanding of the core and fundamental function of fibrillins in oxygenic photosynthetic organisms, we inactivated, individually and in combination, the two genes (*sll1568* and *slr1024*) that encode fibrillin in the cyanobacterium *Synechocystis* PCC6803. Insertional inactivation of either one or both fibrillin genes did not noticeably affect the rate of photoautotrophic growth for *Synechocystis* cultures grown under low irradiance (LL; 12 $\mu\text{E m}^{-2} \text{s}^{-1}$). Under high light (HL; 150 $\mu\text{E m}^{-2} \text{s}^{-1}$), however, each of the three mutants grew more slowly than the wild type (WT), with a reduction in cell content of chlorophyll, and a marked increase in myxoxanthophyll and zeaxanthin, two carotenoids that are considered to be indicators of high light stress. The phenotype of the double knockout mutant was more extreme than either of the single mutants, with this strain exhibiting an elevated myxoxanthophyll content even when grown under LL. Electron micrographs of the *slr1024* mutant and the *slr1024/sll1568* double mutant showed a number of large and unusual inclusion bodies not seen in the WT, although the morphology and number of carboxysomes appeared normal. Immunoblots of a mutant engineered to produce an epitope-tagged Sll1568 polypeptide revealed that this fibrillin migrates in SDS-PAGE with a larger apparent molecular weight for cultures grown under HL compared to that for cultures grown under LL, suggesting that Sll1568 is modified in some way or is covalently bound to an accessory protein under conditions of HL stress. The possible roles of fibrillins in cyanobacteria will be discussed.

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AN UNUSUAL FORM OF GROWTH IN *PORPHYRA*: VEGETATIVE PROPAGATION OR A FORM OF PERENNATION?

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The genus *Porphyra* (Bangiales, Rhodophyta) is one of the major crops in seaweed aquaculture. In 2005, 1.3 million metric tons were produced and valued at 1.4 billion USD. It is a highly appreciated seaweed due to its nutritional properties and especially for its use in the Japanese delicacy - sushi. In relation to other Rhodophyta, also produced through aquaculture, *Porphyra* has an important disadvantage. The species of the genus *Porphyra* do not reproduce vegetatively and are ephemeral. Therefore, the production of *Porphyra* always requires the completion of its life cycle. This makes its production very costly and highly sophisticated. This presentation reports our findings of an unusual form of growth in *Porphyra dioica*. The individuals of this species form “new” bladelets in the basal parts of the original blades (which were originated via conchospores). The bladelets are formed at an early stage in the development of the original blades, prior to any evidence of sexual maturation. In the laboratory, bladelets were formed in more than 60% of the individuals, in temperature ranging from 5 to 20°C. The bladelets remained small (<1-2 cm) and start to grow only when they were detached from the original blades. Once isolated, the bladelets grew at rates similar to the original blades and attained similar lengths. There was also no significant difference in nutrient uptake, tissue carbon and tissue nitrogen of the bladelets in comparison to the original blades when grown separately. This form of production on new biomass, without the need of sexual or asexual spores, was never described in *Porphyra*. This mechanism may be responsible for the perennation of the population of *P. dioica* throughout the year, even when the environmental conditions are not appropriate for the formation of conchospores. This form of growth may also represent an interesting advantage for aquaculture application of this species

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HIGH RESOLUTION SIMS IMAGING OF CATIONS IN DINOFLAGELLATE CHROMOSOMES

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The basic issues and answers to the challenge of high resolution SIMS imaging microanalysis of biomaterials, discussed at SIMS XV, had already been laid out some 20 years ago [1]. This early discussion and review was based on the pioneering work at the University of Chicago on the development and performance of a scanning ion microprobe (UC-SIM) which made use of a Ga⁺ probe extracted from a liquid metal ion source (LMIS). The benefits of the high brightness of this point ion source in the high resolution imaging of cations will be evident in this presentation which made use of the current upgraded version of the UC-SIM [2]. At SIMS XV, we reported UC-SIM studies of divalent cations in mammalian cell mitosis and *Drosophila* polytene chromosomes. From previous studies [3], Ca and Mg were shown to be essential for chromosome condensation and structural stability. These findings suggested that the interaction of these cations with DNA in both advanced vertebrates and the more primitive Diptera may have played a fundamental role in eukaryotic evolution. The results to be presented here extend the universality of this basic interaction, to the chromosomes of even more primitive eukaryotes, the Protistan Dinoflagellates. Much interest in these chromosomes is motivated by their total lack of histones [4], so that cations can interact directly with DNA to neutralize its residual negative charge, thus allowing chromosome compaction. Our cation SIMS images of the chromosomes of *Gymnodinium mikimotoi* and *Gymnodinium dorsum*, confirm and enrich the results of previous studies by analytical electron microscopy [5]. [1] R. Levi-Setti, Ann. Rev. Biophys. Biophys. Chem. 17 (1988) 325-347. [2] J. Chabala et al., Int. J. Mass Spectrom. 143 (1995) 192-212. [3] Strick, et al., J. Cell Biology 155 (2001) 899-910. [4] P. J. Rizzo, in The Biology of Dinoflagellates, (1987), Blackwell, Oxford, pp.143-173. [5] M. Herzog & M.O. Soyer, Eur. J. Cell Biol. 30 (1983) 33-41.

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CELLULAR LOCALIZATION OF CALCIUM OXALATE INCLUSIONS IN RED AND GREEN ALGAE

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Calcium oxalate deposition is the most common form of biomineralization in embryophytes. Although this mineral appears to be formed less commonly in the algae, it has been detected in a wide variety of green algae and several species of red algae. Calcium oxalate crystals in the algae, as in embryophytes, exhibit several characteristic morphological forms and can provide systematically useful characters. Embryophytes deposit calcium oxalate crystals within an unusual intravacuolar membrane system that develops only in certain specialized cells. In contrast, calcium oxalate localization in the algae is remarkably diverse. The crystals may be scattered through the vacuole (e.g. *Apjohnia laetevirens*); they may be attached on the vacuolar face of the cortical cytoplasm (*Chaetomorpha coliformis*); crystals may be immobile within the cytoplasm layer (*Antithamnion kylinii*) or they may move with streaming cytoplasm (*Callipsyigma wilsonis*); or they may appear in both intracellular and extracellular locations in the same thallus (*Spyridia filamentosa*). The variety of cellular locations in which the algae deposit calcium oxalate crystals suggests that the functions of these biominerals will prove to be likewise diverse.

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HOST-CELL PENETRATION IN *AMOEBOPHRYA* SPP. INFECTING *AKASHIWO SANGUINEA*: INHIBITOR AND ULTRASTRUCTURAL STUDIES

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Amoebophrya spp. is a dinoflagellate that infects and kills other dinoflagellates and is increasingly recognized as an important limit on harmful algal blooms. While substantial effort has been put into studying the ecology of this intracellular parasite, less is known about its cell biology. *Amoebophrya* and its host, *Akashiwo sanguinea*, were used in ultrastructural (TEM) and inhibitor (LM) studies of host cell entry. Free-swimming *Amoebophrya* dinospores show electron dense bodies of unknown function in the cytoplasm. TEM images show *Amoebophrya* adhering to the surface of *A. sanguinea* with the electron dense bodies associated with microtubules oriented towards the host cell. Membrane appears to be associated with the microtubules proximal to the host surface. However, nocodazole pre-treated dinospores were not inhibited from entering the host cytoplasm. Parasite pre-treatment with Cytochalasin D inhibited entry into the host cell. These results imply that the microtubules, if essential for entry, are pre-existing, stable microtubules rather than newly polymerized. The cytochalasin D experiments indicate that microfilaments are involved in penetration through the amphiesma. However, microfilaments have not been clearly demonstrated in *Amoebophrya* during host entry. After entry, some possibly host derived microfilaments have been observed. One explanation is that *Amoebophrya* may use short microfilaments that turn over rapidly, as has been demonstrated in the closely related apicomplexans.

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POLISHING OF MUNICIPAL WASTEWATER EFFLUENT BY AN ALGAL ASSEMBLAGE AND PRODUCTION OF FUEL FROM HARVESTED BIOMASS

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Municipal wastewater effluent (MWE) often contains levels of nitrate, phosphate and metals that exceed regulatory discharge limits. We are developing algal mat cultivation as a polishing stage of the wastewater treatment process to lower concentrations of these components. Algal biomass accumulated in such a process could serve as a renewable energy source. We are therefore also investigating methods to produce biodiesel from extracted lipids and to optimize anaerobic digestion of algal feedstock for methane gas production. A pilot system, consisting of three shallow ~40-L capacity ponds inoculated with *Oedogonium*-dominated mats, began operation in October 2006 on the grounds of Sonoma State University. Water flows by gravity successively through each inclined pond and then into a small onsite garden. Flow of MWE at rates from 0.3 to 2.6 L min⁻¹ resulted in the lowering of nitrate levels by 97% to 24%, with similar declines in phosphate

observed, when measured. Consistent with previous reports, the algal mats were found to accumulate metals; elution of copper and lead from a column packed with powdered MWE-cultivated algal biomass showed concentration factors of approximately 100,000 and 22,000, respectively, relative to the source MWE. We are currently working with the City of Santa Rosa to expand our study to a demonstration-scale project.

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MOLECULAR ASSESSMENT OF HAWAIIAN STREAM PERIPHYTON DIVERSITY USING A UNIVERSALLY AMPLIFYING PLASTID MARKER

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The use of DNA for identification of stream periphyton has the advantage of overcoming many of the limitations associated with morphology-based identification. We previously identified a ~410-nt universally amplifying region of the plastid genome, which is an ideal candidate for multi-species assessments of photosynthesizing organisms. To test the recovery of DNA sequences of this marker from an environmental sample, a stream known to have high benthic algal diversity was chosen as the study site from which to obtain a sample. A pooled periphyton sample was collected from Waiahole Stream (windward Oahu, Hawaii) and total genomic DNA was extracted. Following PCR with universal primers p23SrV_f/r1, amplicons were cloned into *E. coli* and individually sequenced. A total of 147 cloned PCR amplicons were sequenced, of which 144 were determined to be algal (including cyanobacteria), and 3 were bacterial. A neighbor joining tree was constructed from the aligned sequences. Sequences clustered into three distinct groups that corresponded to diatoms, cyanobacteria and green algae, which was consistent with the microscopically observed diversity in this sample. Our preliminary results indicate that the p23SrV primers lend themselves well to photosynthetic diversity recovery from this type of environmental sample.

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A PRACTICAL METHOD FOR IDENTIFICATION AND DESCRIPTION OF MICROBIAL EUKARYOTES USING THE TESTATE AMOEBA *CENTROPYXIS* AS A CASE STUDY

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Traditional descriptions of a number of microbial eukaryotes are usually made in the context of a non-standardized discipline. Assigning types or following standardized rules for description and nomenclature was not common practice in early works, making identification and comparison to recent works laborious and often subjective. Hence impeding advances in building biodiversity knowledge about these organisms. Here we are interested in developing a more precise method in order to facilitate identification and description of microbial eukaryotes. We analyzed twelve nominal taxa of the genus *Centropyxis* Stein, 1857 to compare how traditional taxonomic practices conflict with contemporary ones. We collected over 2000 specimens at the Tiete River, Sao Paulo, Brazil, and made morphological measurements of discreet and continuous characters using a compound microscope and a Scanning Electron Microscope; ecological data was observed regarding habitat exploration. These datasets were analyzed and compared to previous literature in order to verify the consistency of described species, varieties and forms. We encountered transitional forms that undermine the distinctions stated for three species and nine varieties. Moreover, a number of problems in the traditional descriptions for these organisms—namely lack of types, non-standardized descriptions and the existence of several infrasubspecific taxa—are incongruent with modern surveys. We suggest an explicit and standardized taxonomic practice in order to enhance our taxonomic concepts for microbial eukaryotes, that relies on a comprehensive listing of all taxa that as been described under the same or different names. This differs from a common revision in that it relies on the usage of names and concepts implied by the authors instead of typification. This will allow advances in describing the biodiversity of microbial eukaryotes and more precise inferences for studies in related areas.

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A NEW BENTHIC/EPIPHYTIC *PROROCENTRUM* SPECIES FROM THE WESTERN NORTH ATLANTIC?

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A benthic/epiphytic *Prorocentrum* believed to be an undescribed species was observed from the littoral zone of the western North Atlantic between the coast of Maine (44°30'N) and Rhode Island (41°20'N), at temperatures between 19 and 23 °C. This possibly new species resembles *P. mexicanum* in its gross morphology (28-31 µm L x 19-23 µm W) and with its prominent apical spine, but displays a pronounced cuneiform indentation into the middle of the right valve. The surface of the theca is smooth with pores of two sizes, the larger pores being arranged in a radial pattern. The ovoid nucleus is posterior and the chloroplasts form a ramified to lacy digitation. Analysis of molecular data may provide additional clues necessary to resolve the phylogenetic relationships between this and other *Prorocentrum* species.

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MOLECULAR EVIDENCE FOR *CHONDROPHYCUS POITEAUI* VAR. *GEMMIFERUS* COMB. NOV. (CERAMIALES, RHODOPHYTA) FROM THE MEXICAN CARIBBEAN. IMPLICATIONS FOR THE TAXONOMY OF THE *LAURENCIA* COMPLEX

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Molecular studies were carried out on *Chondrophyucus gemmiferus* and *C. poiteaui* (Rhodomelaceae) from the Mexican Caribbean. These species are morphologically related, but differ mainly in the presence of apiculate projection of the epidermal cells near the apex of branches. Both species belong to *Chondrophyucus* by having two periaxial cells by each axial segment and a right-angle arrangement of the tetrasporangia, but share in common characteristics among the *Laurencia* species such as presence of secondary pit connections between adjacent epidermal cells. The phylogenetic position of these species is inferred in this study by analysis of chloroplast-encoded *rbcL* gene sequences of 21 taxa, using two Rhodomelaceae and two Ceramiaceae as outgroups. The results obtained corroborate the taxonomy of *Laurencia sensu lato* which comprises the genera *Laurencia*, *Chondrophyucus* and *Osmundea*, and indicate that *rbcL* provides sufficient phylogenetic signal to study the intergeneric and interspecific relationships within the complex. In spite of this, relationships within the clade formed by *C. gemmiferus* and *C. poiteaui* have not been resolved by any analysis as a result of the low level of genetic variation between their *rbcL* sequences (0.01-0.02%). Considering this and the morphological similarities, the following nomenclatural proposal is made: *Chondrophyucus poiteaui* var. *gemmiferus* (Harvey) comb. nov

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MARINE TUBE-FORMING DIATOMS AND THEIR COHABITANTS: A FLORISTIC SURVEY OF CANADIAN WATERS USING DNA BARCODING

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Diatoms are ubiquitous single-celled algae that are commonly identified by the fine structure of their silica valves. Unfortunately, many of the morphological characters used to identify diatoms, including valve shape, pore occlusion, and hollow versus solid spines are qualitative. This can be especially a problem when compiling data across large geographical regions or between various authors. DNA barcoding is a molecular technique that uses sequence comparisons of the *coxI-5'* region of the mitochondrial genome to distinguish species and has been used successfully to identify species of Rhodophyta. My project will use DNA barcoding to identify species of marine tube-forming diatoms and document their distribution and biogeography in Canada. Clonal cultures of these diatoms will be established and used to develop primers for barcoding and a protocol for single cell extraction, which will facilitate analyses of environmental samples and determine the presence/absence of tube-forming diatoms collected from a variety of habitats in the Bay of Fundy. In

addition, we will examine evolutionary relationships among species of the genera *Berkeleya*, *Parlibellus*, and *Navicula* (specifically the tube-forming species) using small subunit ribosomal DNA. Preliminary results for this research program will be presented.

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MORPHOLOGICAL AND MOLECULAR STUDIES ON THE PEYSSONNELIACEAE FROM VANUATU AND SOUTHEASTERN AUSTRALIA

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Owing to the crustose habits and tenacious attachment of most of its members, the Peyssonneliaceae is one of the most under-collected and poorly known of the red algal families. Littered with species and genera of uncertain taxonomic status, the family itself only tentatively resolves as sister to other families of the order Gigartinales in which it is currently positioned. A thorough study of its ordinal position and an evaluation of the anatomical characters indicative of monophyletic species and genera is overdue, particularly in view of its virtually cosmopolitan presence and large numbers of species. I began such a project in the course of marine macroalgal surveys of Vanuatu, an 83-island archipelago in the south Pacific with the most poorly documented algal flora of the region. Collections made over two years have included a high diversity of peyssonnelioid algae representing some four genera and at least 14 species on present anatomical criteria. Supplementing the survey are a number of species and genera from southeastern Australia, many of which show particular morphological links to Vanuatuan taxa. Six of the seven peyssonnelioid species monographed by Womersley are being analyzed, and preliminary work indicates that several new species will need to be described from both regions. Phenetic analyses of Vanuatu and Australian *Peyssonnelia*, *Polystrata* and *Metapeyssonnelia* spp. indicate the presence of three major and seven minor anatomical groups, each defined by unique characters that will be outlined and discussed. These characters do not, however, adequately establish species boundaries within the largest and most poorly resolved group, the one containing plants of *Peyssonnelia rubra*-type anatomy and to which the highly variable and widely reported *Peyssonnelia inamoena* belongs. X-ray diffraction analyses indicate the presence of both aragonite and calcite phases of CaCO₃ in *Polystrata dura* and *Peyssonnelia calcea*, although it is generally held that the Corallinales is the only order of calcite producing red algae. Barcoding and phylogenetic analyses are currently underway, the preliminary results of which will be presented.

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CHARACTERIZATION OF A GREEN ALGA ISOLATED FROM A HUMAN INFECTION

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Although relatively rare, green algal infections of human and animal tissues are occasionally reported. In none of these cases has the agent been described or identified taxonomically. Here we describe and identify a green alga recently isolated from gangrene tissue of the 4th and 5th toes of a diabetic female hospital patient. Axenic cultures of the alga were prepared and cultured routinely in autotrophic medium. Physiological characteristics, morphology and DNA sequence data were then used to characterize it. The alga consists of green spherical non-flagellate unicells, 2.7 - 13.5 µm in diameter, surrounded by a thick, well-defined sheath and containing a single bowl-shaped chloroplast. No pyrenoid is present. Asexual reproduction is through multiple autospores produced within the parental cell wall. DNA (18S rDNA and ITS) sequence analyses indicated that the alga is closely related to *Chlorella saccharophila*, and is much less closely related to the colorless chlorophyte *Prototheca*. This *Chlorella* grows both heterotrophically in darkness and autotrophically in light. D-glucose considerably enhanced the growth rate in light or in complete darkness. The alga could be cultured in undisturbed culture flasks at pH values ranging from 2 to 9. Cultures grew rapidly at 25 °C and somewhat slower at 30 °C. Cultures gradually ceased growing and died within about 10 days after transferring to 37 °C. These results suggest that this strain may not normally invade tissues, but instead can become established and grow on previously infected exposed tissues of body extremities that are exposed to a contaminated source of soil or water.

ANALYSES OF THE UBIQUITOUS SPECIES *CHARA BRAUNII* (CHARALES) IN JAPAN, BASED ON THE MORPHOLOGY, CHLOROPLAST AND NUCLEAR DNA SEQUENCES

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Chara braunii is a unique charalean species that grows in various aquatic habitats from shallow water in paddy fields to the bottom of deep lakes. A large number of infraspecific taxa have been proposed within this species because of its high morphological variability. In order to elucidate the natural existence of *C. braunii*, we carried out molecular phylogenetic analyses and quantitative measurements of morphological characters used for infraspecific taxa, based on 89 samples collected from various localities in Japan including paddy fields and lakes etc. The chloroplast DNA (cpDNA) sequence data from the *rbcL* gene and the inter-genic spacer regions between *atpB* and *rbcL* genes (3154 bp) demonstrated two robust clades (groups A and B). Samples of group A were mainly collected from unstable and shallow aquatic environments such as paddy fields, while those of group B were mainly composed of samples found in comparatively large aquatic environments such as lakes or ponds. Groups A and B therefore seem to represent closely related entities that have recently differentiated and adapted to two different aquatic environments. However, the result of the morphological measurements suggested these groups cannot be delineated by the traditional infraspecific diagnosis. In addition, we carried out phylogenetic analyses based on nDNA sequences encoding the transcribed regions of *hsp90* (3241 bp) and *EF-1 α* (2415 bp). Phylogenetic relationships based on both of the nDNA markers were essentially different from those of cpDNA within each of groups A and B, suggesting outcrossings of the monoecious plants of *C. braunii*. One robust discrepancy of the phylogeny between group A and B was found in the *EF-1 α* tree. Therefore, at least one gene flow must have occurred between group A and B, which may not be completely sexually isolated. Tajima's D test based on these three DNA data matrices was performed to test neutrality of the genes. The results indicated that either region of nDNA sequences was evolutionarily neutral, while the cpDNA region was not neutral, possibly affected by natural selection.

OCCURRENCE OF THREE DIFFERENT GENOTYPES OF THE FILAMENTOUS BROWN ALGA *GEMINOCARPUS* (ACINETOSPORACEAE, ECTOCARPALES, PHAEOPHYCEAE) IN ANTARCTICA

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In the marine phytobenthos mesograzers (small herbivorous animals such as amphipods) may be able to shape the structure of whole communities. In the macroalgal flora of Antarctica activity of mesograzers was hypothesized to be responsible for the scarcity of subtidal small filamentous algae which were suspected to escape herbivory by growing mainly as endophytes in larger algae (Peters 2003). Such endophytes usually lack diagnostic morphological characters and we are developing molecular tools for their identification.

Geminocarpus Skottsberg is such a genus of filamentous epiphytic and endophytic brown algae endemic to sub-Antarctic and Antarctic regions. Two species are formally recognized: *G. geminatus* (Hooker et Harvey) Skottsberg (type locality Cape Horn) and *G. austrogeorgiae* Skottsberg (type locality South Georgia). The geographic distributions of these two species are not clear, indeed some authors question whether *G. geminatus* is present in Antarctica - suggesting that all Antarctic *Geminocarpus* belong to *G. austrogeorgiae*. However, we have previously documented that morphological forms matching *G. geminatus* do occur in Antarctica. We found this putative *G. geminatus* commonly epiphytic on second-year, senescing *Desmarestia antarctica* during November and early December. We have started to use molecular data from replicate samples to resolve the problem of species identity and diversity in Antarctic *Geminocarpus*. So far sequences are only known from *G. austrogeorgiae*, a species which was found endophytic in the large Antarctic brown alga *Desmarestia menziesii*. The results suggest that in addition to *G. austrogeorgiae* two more genotypes (possibly species) of *Geminocarpus* occur in West Antarctica.

CURRENT PROGRESS TOWARDS AN ALGAL HETEROKONT TREE OF LIFE

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Currently, 14 classes of heterokont algae are recognized, and they include over 100,000 described species (as many as one million total species has been estimated by experts). Common members include brown seaweeds and the diatoms. Remarkably, despite two centuries of light microscopic study, 50 years of electron microscopic study, and 20 years of molecular investigations, phylogenetic relationships among the 14 classes remains unknown. This project is estimating relationships among the heterokont classes by generating two large molecular data sets. First, DNA sequences of seven nuclear, mitochondrial, and chloroplast genes from 270 heterokont algal species and 30 non-photosynthetic relatives are being gathered. Second, entire plastid genomes from 30 species will be sequenced. During the first nine months of this project we have devoted most of our time to growing cultures and performing a pilot study of 30 taxa. This pilot study is being performed to make a final selection of the seven genes (3 nuclear, 2 mitochondrial, and 2 plastid genes) for expanded taxon sampling. Our preliminary studies are initially exploring a larger number of potential genes, including the nuclear genes SSU rRNA, actin, heat shock protein 70 and 90, alpha and beta tubulin, the plastid genes *rbcL*, *psbC*, *cfxQ*, and the mitochondrial genes *nad2*, 4, and 5. In addition, we have developed a fosmid cloning approach for plastid genome sequencing. This approach has enabled the sequencing of plastid genomes within several lineages of algal heterokonts that have not yet been analyzed.

A MULTIGENE APPROACH TO RECONSTRUCTING EUGLENOID SYSTEMATICS

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Bayesian and Maximum Likelihood analyses, utilizing a combined dataset developed from the nuclear small and large subunit (SSU & LSU) rDNA and chloroplast SSU rDNA gene sequences, were used to resolve relationships and clarify generic boundaries among 87 strains of plastid-containing euglenophytes representing 9 genera. Taxa from nearly all of the major photosynthetic genera, *Phacus*, *Lepocinclis*, *Colacium*, *Trachelomonas*, *Strombomonas*, *Monomorphina* and *Cryptoglana* formed well-supported monophyletic clades. Taxa from the genus *Euglena* sorted into three clades. The majority of the species sampled in the genus *Euglena* grouped into a single large, well-supported clade. However, *Euglena proxima* appeared as an independent divergence sister to the larger *Euglena*, *Trachelomonas*, *Strombomonas*, *Monomorphina*, *Colacium* and *Cryptoglana* clade. Within this larger clade, a small group of *Euglena* species (*E. anabaena*, *E. exilis*, *E. caudata* and *E. clavata*) formed a clade sister to the *Monomorphina/Cryptoglana* clade. The phylogenetic relationships inferred from the molecular data were well-supported by morphological characters. All analyses supported the monophyly of *Colacium*, *Trachelomonas* and *Strombomonas*. These taxa all share the ability to produce copious amounts of mucilage to form loricas or mucilaginous stalks. Furthermore, chloroplasts with inner projecting pyrenoids are found only in these genera. *Monomorphina* and *Cryptoglana*, both of which contain small, flattened unicells with a single chloroplast and large, plate-like paramylon grains, formed well-supported sister clades. *Phacus* and *Lepocinclis*, both of which have numerous small discoid chloroplasts without pyrenoids and lack peristaltic euglenoid movement (metaboly), also formed a well-supported monophyletic lineage. Like taxa in *Phacus* and *Lepocinclis*, taxa in the new genus *Discoplastis* contained small discoid plastids lacking pyrenoids, but they are not rigid and possess the ability to undergo

metaboly. With the exception of *E. proxima* and the taxa in the *E. anabaena* group, all of the major genera have now been clarified and species level analyses and descriptions are underway.

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PHYLOGENY OF THE EUGLENALES INFERRED FROM CHLOROPLAST SSU AND LSU RDNA SEQUENCES

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We analyzed the chloroplast SSU and LSU rDNA from 119 strains in most genera of photosynthetic euglenoids including three strains of the Eutreptiales as outgroup species. A total of 121 taxa including two previously published sequences, *Euglena gracilis* and *E. longa*, were used for phylogenetic analyses. Bayesian analysis suggested that photosynthetic euglenoids are divided into five major clades; 1) *Discoplastis*, *Phacus* and *Lepocinclis* clade, 2) *Colacium* clade, 3) loricate genera *Trachelomonas* and *Strombomonas* clade, 4) *Cryptoglena* and *Monomorphina* clade, 5) two *Euglena* clades. The *Discoplastis*, *Phacus* and *Lepocinclis* clade occupied a basal position among members of the order. The genus *Discoplastis* branched first, and then *Phacus* and *Lepocinclis* emerged as sister groups. Subsequently the genus *Colacium* was well supported as a monophyletic clade. The loricate genera *Trachelomonas* and *Strombomonas* formed a monophyletic clade with high support values. The genera *Cryptoglena* and *Monomorphina* also formed a well supported monophyletic clade. However, the genus of *Euglena* clade was divided two clades. First clade was located at the top of tree and composed of most species of subgenus *Euglena* and *Calliglena*. The other *Euglena* species, *E. anabaena*, *E. caudata*, and four unidentified species, were not formed a well defined clade, but showed sisterly relationships with two big groups, most *Euglena*, *Monomorphina* and *Cryptoglena* group and loricate group.

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COMPARISONS OF INSHORE AND OFFSHORE ARCTIC MARINE PICOEUKARYOTES

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Arctic Seas were thought to be dominated by large phytoplankton but more recent studies tend to show the major importance of picoplanktonic cells ($\leq 3 \mu\text{m}$) over much of the year in these perennially cold environments. Our earlier work suggested that picoeukaryote diversity is high across Arctic marine regions, with both geographical and depth differences in community structure. As part of a marine microbial survey in the Canadian Arctic Beaufort Sea region we analyzed samples collected from two sites; a shallow coastal station in Amundsen Gulf (64 m) and a deep station in the Canada Basin (2503 m) in September 2005. Samples from six discreet depths were used to investigate marine picoeukaryote species diversity by way of Denaturing Gradient Gel Electrophoresis (DGGE) and clone libraries based on the 18S rRNA gene. We further estimated the abundance of several key picoeukaryotic clades down the water column using specific primers and Q-PCR. Samples from the characteristic Arctic Ocean deep chlorophyll maximum and the nitracline revealed higher diversity than surface or deeper samples. These samples were selected for detailed analysis and used to generate clone libraries. The diversity indices from the two study sites were of the same scale. The arctic ecotype of the small prasinophyte, *Micromonas* sp., was ubiquitous but varied in abundance. Other diverse protist clades varied in representation, these included marine stramenopiles (MAST) and dinophyceae clades. Finally, Acantharian and Rhizarian clades were only recovered from the deep water site.

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PHYLOGENY AND TAXONOMY OF THE HAWAIIAN *LAURENCIA* COMPLEX (RHODOMELACEAE, RHODOPHYTA)

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Most of the Hawaiian *Laurencia* species are common in the intertidal and shallow subtidal, and sixteen species are currently recognized. Recently, a cladistic analysis of the *Laurencia* complex resulted in the recognition of another genus, *Palisada*, within *Chondrophycus*. Consequently, currently there are four genera in the *Laurencia* complex, three of which (*Chondrophycus*, *Laurencia* s.s., and *Palisada*) are known from Hawaii. In

order to examine the phylogenetic position of the Hawaiian species, we conducted a molecular phylogenetic analysis based on *rbcL* sequences, adding samples from the main Hawaiian Islands (Hawaii, Kauai, Lanai, Maui, Molokai, and Oahu) as well as samples from Australia, Costa Rica and Spain to previously published sequences. As a result, the monophyly of *Osmundea* was supported with high bootstrap values, and *Chondrophyucus* samples were recognized in two independent clades. One of the *Chondrophyucus* clades, which includes the type species, *C. cartilagineus* (Yamada) Garbary & Harper, was highly supported; however, the second clade, which may correspond to *Palisada*, was weakly supported. The phylogenetic position of *Laurencia* s.s., on the other hand, appears to be unstable, which means that this lineage is potentially monophyletic, paraphyletic or polyphyletic, depending on taxon sampling. This might be caused by the low resolution at the internal nodes near the basal part of the *rbcL* tree (in order to further investigate this problem, we at present are carrying out an 18S rDNA analysis). Conclusively, our *rbcL* tree showed that the Hawaiian species belong to three major groups, excluding *Osmundea*. We will discuss the taxonomy of the Hawaiian *Chondrophyucus* lineage.

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PHYLOGENETIC RELATIONSHIP OF VARIOUS *CERAMIUM* (CERAMIACEAE, RHODOPHYTA) FROM THE NORTH PACIFIC INFERRED FROM RBCL DNA SEQUENCE DATA

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To test the monophyly of the genus *Ceramium* in the North Pacific and infer its phylogenetic inter- and intrarelationships, an *rbcL* dataset was compiled from 20 taxa. Investigated species with complete cortication include *C. kondoi*, *C. boydenii*, *C. codicola*, *C. horridum*, *C. pacificum*, *C. johnstonii*, *C. japonicum*, and an undescribed species from Mexico. Species with incomplete cortication include *C. aduncum*, *C. affine*, *C. cimbricum*, *C. clarionense*, *C. equisetoides*, *C. gardneri*, *C. inkyuii*, *C. paniculatum*, and an undescribed species from Mexico. Species with intermediate cortication include *C. californicum* and *C. interruptum*. The *rbcL*-based phylogeny indicates that the North Pacific species placed in *Ceramium* do not belong in a single genus as the *C. aduncum* clade does not cluster with the remaining *Ceramium* clades in a global *Ceramieae* phylogeny. New information about types of cortication and tetrasporangial development is provided to further characterize the species groups and the genus *Ceramium*.

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MOLECULAR ADAPTATION OF GAMETOLYTIC, MATRIX METALLOPROTEINASE 1 (MMP1) GENE IN *CHLAMYDOMONAS* STRAINS

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Matrix metalloproteinases (MMPs) are zinc-dependent endopeptidases, widely distributed in both eukaryotes and prokaryotes. *Chlamydomonas* has been used as a model organism to infer the molecular mechanisms of MMP activity in algae. Different genes of *Chlamydomonas* the MMP gene family are associated with different functions such as vegetative (MMP3), gametic (MMP1), and zygotic (MMP2) cell wall lysis. Despite the identification and characterization of different MMPs that are involved in different functions in *Chlamydomonas*, little is known about the underlying genetic mechanism that causes rapid functional diversification of each MMP gene. Using the maximum likelihood-based codon substitution approach, here we report that although the entire coding region of MMP1 is subjected to purifying selection, certain amino acids (~ 0.8%) are shown to be under the influence of positive Darwinian selection. These positively selected amino acid sites might have caused rapid functional diversification among these *Chlamydomonas* strains.

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RED ALGAL ROGUE ACROCHAETES: *RHODOCHORTON MEMBRANACEUM* AND *R. SUBIMMERSUM* ARE ALLIED TO THE PALMARIALES

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The red algal Acrochaetiales, Colaconematales, and Palmariales form a complex united by similarities including pit plug (proteinaceous cores that close incomplete wall deposition between cells) ultrastructure, and relatively simple reproductive features. An eventful taxonomic history has seen some former anomalous members of the Acrochaetiales, specifically *Meiodiscus spetsbergensis* (formerly *Rhodochorton spetsbergense*) and *Rhodothamniella floridula*, transferred to the Palmariales based on morphological, biochemical (phycoerythrin-type), and life history (monosporangia and carposporophyte stage absent) attributes. These transfers were confirmed by the addition of subsequent molecular data. Here we discuss other rogue members of the Acrochaetiales - two species of *Rhodochorton*: *R. membranaceum*, an endozoic acrochaete; and *R. subimmersum*, an epi/endophytic species - and the nature of their morphological, biochemical, life history, and molecular affinities to the Palmariales. Molecular analyses employed the nuclear large-subunit ribosomal DNA (LSU), a suitable region for distinguishing red algal phylogeny at the ordinal level and below, and the more variable mitochondrial 5' *cox1* DNA barcode, as a species identifier. Preliminary LSU results support the transfer of both these taxa to the Palmariales. The resulting reorganization and changes necessitated in taxonomy at the species, generic, and familial levels are discussed.

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TAXONOMY OF *COOLIA* INCLUDING TWO NEW SPECIES, *COOLIA MINUTA* SP. NOV. AND *COOLIA NOVELLA* SP. NOV. (DINOPHYCEAE)

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Two new benthic dinoflagellate species are described, *Coolia minuta* sp. nov., and *Coolia novella* sp. nov. Cells were identified in Belizean coral reef mangroves raising the known *Coolia* species to 5. Establishment of these species is supported by morphological differences and separate phylogenetic analyses of the small subunit rDNA genes. Sequences included in the phylogenetic analyses were derived from a type cultures for *C. monotis* available in GenBank and from single cell isolates of *C. tropicalis*, *C. minuta* and *C. novella* isolated from the central lagoon reef system of Belize. The two new *Coolia* species were distinguished from other *Coolia* species: cells of *C. minuta* by small size, narrow wedged-shaped 1' plate, trapezoid 3' plate, and a short apical pore; whereas, *C. novella* by medium size, broad 1' plate, and crescent-shaped 3' plate, and a short curved apical pore. Morphological difference, however, are only discernible using scanning electron microscopy, and are not readily apparent using light microscopy. This precludes accurate identification of *Coolia* species difficult using only light microscopy thereby complicating distributional and ecological studies. Whether these new species produce ciguatoxins is not currently known.

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THE SUBAERIAL ALGAE: A CASE FOR MORPHOLOGICAL CONVERGENCE?

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While assessing the biodiversity and systematics of subaerial algal microchlorophytes from African tropical rainforests (Gabon), several examples have been found showing similar thalli morphologies. Comparable examples have been found in other regions. Three main types of thalli characterize the morphology of subaerial algae: 1) unicellular, the most widespread in terms of the number of species; 2) sarcinoid (packets formed by a small number of cells), characteristic of one of the most common alga in the world (*Desmococcus*); and 3) uniseriate, found in a relatively limited number of species. These similarities encompass not only thallus morphology, but also growth pattern, pigmentation, and other biochemical and cytological adaptations. However, it has been shown that morphology and reproductive features do not reflect phylogenetic patterns in subaerial microchlorophytes. Morphological convergence is the pattern of long term change in similar directions among remotely related lineages (Starr, Cecie and Taggart, 1995). This subaerial algal community may be interpreted as a case for morphological convergence. Ecological factors may play a significant role imposing particular constraints on living organisms such as the subaerial microchlorophytes. Furthermore, behind these very limited number of shared morphologies underlies a great deal of genetic diversity. We present specific cases of subaerial algal taxa from tropical rainforests where simplification of the

thallus has occurred.

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MOLECULAR CHARACTERIZATION OF THE RED ALGAL GENUS *SCINAIA* (SCINAIACEAE, NEMALIALES) FROM THE AZOREAN ARCHIPELAGO WITH MORPHOLOGICAL OBSERVATIONS ON *S. INTERRUPTA*

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Two species of *Scinaia* that have been reported to co-exist in the Azores, *S. furcellata* and *S. interrupta*, are commonly confused with one another. Because *Scinaia interrupta* has been incompletely described morphologically, its segregation inside the *S. carnosa*-group *sensu* Huisman 1986 had not been confirmed. In the present study recently collected specimens from the Azorean Archipelago (*S. interrupta* and two non-identified species), the Gulf of Mexico and historical vouchers of *Scinaia* housed in The Natural History Museum (BM) were investigated. Chloroplast-encoded *rbcL* sequence analysis was conducted from silica gel-preserved specimens belonging to different populations. The *rbcL* tree confirmed the occurrence of three different species of *Scinaia* for the Azores and one from the Gulf of Mexico. The presence of rhizoidal filaments is the only identifiable character of *S. interrupta* inside the *S. carnosa*-group. Morphological characterization of the other species is still in progress

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CLARIFICATION OF THE RED ALGAL GENUS *PEYSSONNELIA* IN THE GULF OF MEXICO, WITH A PROPOSAL FOR A NEW RED ALGAL ORDER BASED ON THE PEYSSONNELIACEAE

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The crustose genus *Peyssonnelia Decaisne* is a taxon of great ecological significance, with some species involved in the establishment of rhodoliths. Comparative morphological and molecular analyses demonstrate a greater diversity of peyssonneloid species than was previously reported. In chloroplast-encoded *rbcL*- and nuclear LSU rDNA-based trees, species referred to as *Peyssonnelia* in the literature do not group together, but are scattered among other genera that were either previously or currently placed in the Peyssonneliaceae. Two newly reported genera for the Gulf of Mexico, *Polystrata* and *Metapeyssonnelia*, are excluded from the family, and together with a third clade are nested inside the Rhizophyllidaceae of the Dumontiaceae-complex. The Rhizophyllidaceae is newly reported for the Gulf of Mexico, with six species. The number of distinct species of Peyssonneliaceae now present in the Gulf of Mexico has increased from 6 to 21. Species placed in *Cruoriella* and *Cruoriopsis* belong in the Peyssonneliaceae. New combinations are being proposed to accommodate known and new species in *Cruoriella*, and in two formerly monotypic genera, *Sonderopelta* and, provisionally, *Riquetophycus*. The Peyssonneliaceae form a monophyletic assemblage that cannot be maintained in the Gigartinales and thus constitutes a new order, unrelated to the cluster of families centered around the Halymeniaceae of the Cryptonemiales (=Halymeniales), or the Gigartinaceae of the Gigartinales.

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NEW INSIGHTS IN THE RED ALGAL ORDER RHODYMENIALES, WITH SPECIAL EMPHASIS ON TAXA FROM THE GULF OF MEXICO

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Recent dredging expeditions throughout the Gulf of Mexico (NW: offshore Louisiana and Texas, SW: Gulf of Campeche, Mexico; SE: vicinity of the Dry Tortugas, FL; NE: vicinity of the Florida Middlegrounds, FL) at depths between 45-90 m have revealed an exceptional species-rich diversity of Rhodymeniales. Chloroplast-encoded *rbcL* sequences were analyzed from more than 120 vouchers of Rhodymeniales from the Gulf, and

worldwide, belonging to the Rhodymeniaceae, Champiaceae, Faucheaceae, and Lomentariaceae. Emphasis is placed on a critical revision of the generic concepts within each family, the proposal for new species descriptions, and the establishment of new or revised range distributions for pertinent taxa.

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EXAMINING THE EUGLENOPHYTE MUCILAGINOUS CLADE WITH EF1 α

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Euglenids have been studied since Antonie van Leeuwenhoek first discovered the world of protists; however, we are still far from understanding them. Although all Euglenids are able to secrete mucilage there are three genera that do this in abundance: *Colacium*, *Strombomonas* and *Trachelomonas*. Euglenids within the genus *Colacium* are the only known colonial euglenids that bind together with strands of bifurcating mucilage secreted from their anterior ends. *Strombomonas* and *Trachelomonas* species secrete mucilage over the entire cell to form a protective lorica with large amounts of iron and magnesium within it. In the case of *Trachelomonas* the lorica is hardened. We examined the validity of this mucilage-secreting clade by using gene sequences of a novel molecular marker, elongation factor 1 alpha (EF1 α). Sequences of EF1 α were analyzed alone as well as in concatenation with other genetic markers, such as ribosomal DNA (rDNA). By comparing EF1 α sequences both within and between those of other Euglenophyte taxa as well as heterotrophic Euglenids we hope to clarify the sister relationships of these three genera.

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AN EXPLORATION OF THE GENUS *GEITLERINEMA* (PSEUDANABAENACEAE) USING A COMBINED MOLECULAR AND MORPHOLOGICAL APPROACH

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One of the most cosmopolitan, yet poorly studied and characterized genera of cyanobacteria is *Geitlerinema* (Pseudanabaenaceae), a freshwater genus that is commonly found in oligotrophic and mesotrophic waters. The diagnostic characteristics of this genus are based upon morphological features, distinguished mainly by the presence of an enlarged apical cell. However, molecular techniques have yet to be employed to test the monophyly of this genus. Several strains of *Geitlerinema* were isolated from Florida and culture strains were obtained from the University of Toronto Culture Collection. Phylogenetic analysis was performed using the 16S rDNA and internally transcribed spacer (ITS) gene sequence data from these strains in combination with information from those strains available in GenBank. Two distinct clades was recovered, whose members were not necessarily clearly discernable by morphological examination. In addition, the use of secondary folding patterns of the 16-23S ITS region and cell ultra structural features (e.g., type of cell division, thylakoid arrangements), along with ecological data, was employed. Several of the strains employed did not match any currently circumscribed species, and are putatively new to science. Through this total evidence approach, we propose that *Geitlerinema* as currently circumscribed is polyphyletic and in need of revision to establish monophyletic lineages.

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CHARACTERIZATION OF A MYCOPHAGOUS AMOEBA-FLAGELLATE ISOLATED FROM A *PHYTOPHTHORA RAMORUM*-INFECTED LESION OF CALIFORNIA BAY LAUREL

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An amoeba-flagellate, termed strain ANN04, was isolated from hyphae of *Phytophthora ramorum* originating from an infected California bay (*Umbellularia californica*) leaf lesion. *P. ramorum*, an oomycete, is the causative agent of "Sudden Oak Death", a disease syndrome currently afflicting forests along the coasts of Central and Northern California. ANN04 was separated from *P. ramorum* by culturing on heat-killed bacteria and the co-culture reestablished on a separate *P. ramorum* strain. Trophozoites (~10 μ m in length) show putative feeding behavior on hyphae and sporangia of *P. ramorum*. Higher densities of trophozoites and cysts

could be observed by co-culturing with an ascomycete, termed strain F1, which was also isolated from the same infected bay leaf lesion as ANN04. Further studies to determine the feeding range of strain ANN04 are underway. Characteristic of *Naegleria* spp., suspension of ANN04 trophozoites into dilute medium induced a temporary flagellated stage. However, to our knowledge, there are no reports of mycophagy by *Naegleria*. To better establish the taxonomic identity of strain ANN04 we will be conducting rDNA and actin gene sequence analysis. The potential application of ANN04 as a biocontrol agent will be discussed in the context of studies by Old and Chakraborty [1] on the apparent role of mycophagous amoebae in suppression of soil-borne *Phytophthora* diseases. [1] Old, K.M. and Chakraborty, S. 1986. Mycophagous soil amoebae: Their biology and significance in the ecology of soil-borne plant pathogens. In: Patterson, D.J., & Corliss, J.O. eds, *Progress in Protistology* 1:163-194.

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GRAZING, GROWTH, AND BEHAVIORAL REACTIONS OF A CILIATE FED *ALEXANDRIUM* SPP: APPARENT LACK OF RESPONSE TO SAXITOXIN

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Harmful algal blooms can cause economic and ecological damage. Ciliates are important grazers of planktonic algae, and may be impacted differently by harmful algal blooms than are other grazers. The bloom-forming dinoflagellate *Alexandrium fundyense* produces saxitoxin (STX) and a number of similar compounds. STX is considered to be a voltage-gated sodium ion channel blocker, but has been shown also to have an effect on calcium channels, which are important in regulation of motility in ciliates. Experiments were carried out with the ciliate *Strombidinopsis* sp. fed two members of the *Alexandrium fundyense* complex, *A. fundyense* and *A. tamarensense*. *A. tamarensense* is considered to be non-toxic or less toxic than *A. fundyense* because it does not produce STX. While both dinoflagellates make other STX-related compounds, *A. tamarensense* produces less of these than *A. fundyense*. The ciliate fed on both species of *Alexandrium*. It survived, but did not grow on *A. fundyense*; however, there was significant mortality in ciliates fed *A. tamarensense*. Behavioral assays showed unexpected differences in reactions to *A. fundyense* and *A. tamarensense* extracts, with avoidance (backwards swimming) being induced by *A. tamarensense* extract but not *A. fundyense*. We are presently developing further the behavior assay and evaluating the effects of calcium channel blockers on motility and grazing in planktonic ciliates. Our goal is to identify the mechanism of toxicity and to assess the role of this interaction in promoting harmful algal blooms.

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ASSEMBLY OF RIBOSOMAL DNA IN *PNEUMOCYSTIS*

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The ribosomal locus is of fundamental importance for understanding the evolution, biology, and pathology of *Pneumocystis*. Studies on the nuclear genes encoding ribosomal RNAs (rDNA) of *Pneumocystis* led to reclassification of *Pneumocystis* as a fungus. Most other fungi contain hundreds of copies of rDNA arranged as head-to-tail tandem repeats separated by no more than a few kb. Quantitative hybridization and PCR have showed that *Pneumocystis* has no more than two copies of the rDNA locus. In the present study, we sequenced a cosmid containing the rDNA locus. The cosmid contained a single copy of the *Pneumocystis* rDNA locus, encoding 18S, 5.8S, and 26S ribosomal RNAs, and no other rDNA was within ~16kb and ~13kb upstream and downstream, respectively. Regions flanking the *Pneumocystis* rDNA contained the following putative orthologues: cell morphogenesis protein (PAG1); NADPH-cytochrome P450 reductase (CprA); 19S regulatory cap region of 26S proteasome subunit 2 (mts4); U3 snoRNP (Utp6p); developmental regulator FlbA; CCCH zinc finger protein; and v-SNARE binding protein. Getting the complete sequence of the rDNA locus and regions flanking it required a combination of shotgun sequencing from the *Pneumocystis* genome and directed sequencing of sections of the rDNA cosmid. We used the original sequence assembly to form a sequencing backbone of 38 kb. We assembled the original cosmid sequence with sequencing reads and their associated chromatograms from the *Pneumocystis* Genome Project. This supplemented and extended the previously

reported cosmid sequence by providing overlapping reads, base call quality scores and chromatograms across the cosmid. The assembly was conducted using the Gap4 assembler (allowing sequence alignments with up to 15% base mismatching). The Gap4 visual assembly tool was used to examine the overlapping reads across the cosmid. The visual interface showed a dramatic bias in the coverage of shotgun reads that correlated with GC content, which showed that physical gaps are caused by poor clonability of AT-rich segments. The *Pneumocystis* Genome Project sequences provided increased base call confidence and validation by adding chromatograms and nucleotide base quality scores to the assembly.

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UPDATED DRAFT ASSEMBLY AND ANNOTATION OF THE *PNEUMOCYSTIS CARINII* GENOME
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Pneumocystis carinii (Pc) presents challenges to genomic sequencing and assembly technologies. The genome is very AT rich (67%) and this led to bias in the shotgun library representation and localized poor coverage. Nevertheless, the genome project produced 100,000 reads and these were assembled into 4272 contigs. Despite multiple attempts to merge contigs using the previously reported iterative assembly, overlapping contigs remained unmerged by this process. The previously reported draft assembly process was difficult to verify due to the iterative nature of the assembly, which lacked underlying read alignments to the contigs. Using the Pc draft assembly as a backbone, we reassembled the contigs using the Gap4 assembler (allowing up to 15% base mismatches). This reduced the previously reported number of contigs from 4272 into 3846, reducing the number of overlapping contigs and collapsing the assembly. We further reduced the number of overlapping contigs by re-assembling the underlying individual sequence reads into the collapsed backbone assembly. The result was a second draft of the Pc genomic assembly containing ~6.7 million sub-telomeric base pairs contained within 3589 contigs. The number of Pc Contigs \geq 10Kb rose from 77 in the iterative draft assembly to 92 contigs in this updated Pc genomic assembly. This second draft of the Pc genome can be visually inspected and biologically verified. By aligning underlying reads to the backbone assembly each sequence alignment and each predicted nucleic acid base may be examined. The underlying chromatograms and base quality scores for each sequence alignment and base call can be inspected. Assembled contigs that contain areas of low sequence depth or poor underlying read quality may be further examined. Areas of low genomic coverage may be biologically examined by PCR amplification. Biologically disproved sequence alignments may be easily separated. Alternatively, unmerged overlapping contigs are difficult to identify and biologically verify. A homology based annotation was conducted on the re-assembled genomic contigs using the KEGG-KASS annotation server.

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WHY DO PHYTOPLANKTON MAKE SUCH A FUSS ABOUT CARBON DIOXIDE?

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This presentation reviews our present understanding, based on work in many laboratories, of how phytoplankton acquire inorganic carbon and assimilate it into organic carbon. Considerations of the Redfield Ratio (106C:16N:1P) and the ratio of total inorganic carbon to total available nitrogen and to available phosphorus suggest that inorganic carbon is not the nutrient limiting phytoplankton growth in most of the ocean and some inland waters. This conclusion holds when other potential limiting nutrients, e.g. iron, are considered in the context of the Extended Redfield Ratio. The kinetic characteristics of various algal Rubiscos, the speciation of inorganic carbon in seawater, and the diffusion characteristics of carbon dioxide in moving from the bulk medium to Rubisco, can be used to rationalise the very widespread occurrence of inorganic carbon concentrating mechanisms (CCMs) in phytoplankton. The extent of CCM expression, and the affinity for inorganic carbon in photosynthesis and growth by cells, shows acclimation as a function of the availability of inorganic carbon, nitrogen, phosphorus, iron and photosynthetically active radiation. The occurrence of

CCMs, and the acclimatory responses of the CCMs, in today's ocean will be considered in the context of the evolutionary origin of CCMs, in the context of a comparison with the means of acquisition and assimilation of other nutrients. The presentation concludes with a consideration of the possible effects of environmental change on inorganic carbon acquisition and assimilation. Acknowledgement: Funding for work on the acquisition and assimilation of inorganic carbon by phytoplankton from the Natural Environment Research Council (UK) is gratefully acknowledged.

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SIZE MATTERS: MACROEVOLUTIONARY PATTERNS IN MARINE DIATOMS AND DINOFLAGELLATES

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The size structure of marine primary producers strongly influences food web interactions and the biogeochemical cycling of carbon. A macroevolutionary record of the size of the dominant fossilized marine diatoms and the dinoflagellates indicates that the median size of the marine diatoms and dinoflagellates is strongly correlated with climate, especially deep ocean temperatures over the Cenozoic. In addition there is some evidence that changes in phytoplankton assemblage size structure is coupled with changes in the size structure of some zooplankton groups and higher trophic levels. This indicates that long-term climatic changes may have shaped the size distribution of primary producers in the ocean and potentially altered both the rate of export of carbon in the ocean and marine food web structure.

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A 2 YEAR LONGITUDINAL STUDY OF *CRYPTOSPORIDIUM* SPECIES AND GENOTYPES IN DAIRY CATTLE

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In this, the first long term longitudinal study of cryptosporidiosis in cattle, 30 pure-bred Holstein female cattle on a dairy farm in Maryland were examined consecutively at weekly, biweekly, or monthly intervals from 1 week to 24 months of age for the presence of *Cryptosporidium* oocysts. Feces were sieved and subjected to CsCl₂ density gradient centrifugation to concentrate oocysts. The presence of oocysts was determined by both immunofluorescence microscopy and PCR/gene sequence analysis of the 18S rRNA gene. Prevalence was higher by PCR than by IFA. All 30 calves shed *Cryptosporidium* oocysts at some time during the study. Of 990 fecal specimens collected, 172 (17%) contained oocysts. Differences in prevalence were found based on the age of the animals. A higher prevalence was detected in pre-weaned calves (less than 8 weeks of age) (38.3%) than post-weaned calves (3-12 months of age) (18.2%) or heifers (12-24 months of age) (2.2%). The species/genotypes of *Cryptosporidium* that were identified included *C. parvum*, *C. bovis*, *C. andersoni* and *Cryptosporidium* deer-like genotype. The prevalence of each of these species and this genotype was age related: *C. parvum* was the most prevalent species in pre-weaned calves whereas *C. bovis* and *Cryptosporidium* deer-like genotype were more prevalent in post-weaned calves and heifers. *Cryptosporidium parvum*, the zoonotic species, was detected in all thirty pre-weaned calves, confirming previous cross-sectional studies from dairy farms.

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GENETIC DIVERSITY OF A MALARIA PARASITE, *PLASMODIUM MEXICANUM*: CHANGES IN DIVERSITY OVER TIME AND SPACE AND EFFECTS ON PARASITE LIFE HISTORY TRAITS

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Within the vertebrate host, infections of a malaria parasite (*Plasmodium*) could include a single genotype of cells (single-clone infections) or two to several genotypes (multiclone infections). Clonal diversity of infection plays an important role in the biology of the parasite, including its life history, virulence, and transmission. We determined the clonal diversity of *P. mexicanum*, a lizard malaria parasite at a study region in northern California, using variable microsatellite markers, the first such study for any malaria parasite of lizards or birds. This study examined two aspects of malaria genetic diversity: the overall diversity of the parasite

metapopulation at our field site in Hopland, CA and the effect diverse infections have in the alteration of parasite life history traits. The prevalence of multiclonal infections (50 - 88% of infections), and measures of genetic diversity for the metapopulation over a 10-year period indicated a substantial overall genetic diversity. Comparing years with high prevalence (1996 to 1998 = 25 - 32% lizards infected), and years with low prevalence (2001 to 2005 = 6 - 12%) found fewer alleles in samples taken from the low prevalence years, but no reduction in overall diversity ($H = 0.64 - 0.90$ among loci). The high prevalence of multiclonal infections is interesting in terms of both the transmission biology and the life history traits of the parasite. Multiclonal infections are presumed to alter parasite life history traits in favor of rapid proliferation due to competition for resources, including transmission. We examined variations in parasitemias and growth rates in induced experimental infections over a three-month period. While the proportion of asexual parasites and total parasitemias did not differ between multi and single-clone infections ($P > 0.05$), we have found that multiclonal infections have a higher rate of gametocyte (transmission stage) production ($P = 0.02$) and, thus, a higher overall gametocytemia ($P = 0.04$), supporting the theory that diverse infections compete for transmission opportunities.

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LABYRINTHULOMYCETES DIVERSITY IN TEMPERATE ESTUARIES

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The Labyrinthulomycetes are ubiquitous marine osmoheterotrophic fungoid organisms that have been studied over the last 10 years mainly because of their relationship to diseases of sea grass meadows (eel-grass or turtle grass) or invertebrates (clams) and their biotechnological potential (polyunsaturated fatty acid production). However, their diversity in natural environments has not yet been adequately explored. The aim of this project was primarily to compare how many different Labyrinthulomycete DNA sequences can be obtained from muddy sediments collected in Peconic Bay and sandy sediments collected in Port Jefferson Harbor (both sites on Long Island, NY) with three PCR primer sets (LabyY-PrimA, LabyA-LabyY and QPXF-R2), and to examine the phylogenetic relationships among these sequences and all the Labyrinthulomycete 18S rDNA sequences found in GenBank. The amplicons produced by LabyA-LabyY were too short (~450 bp) to provide confidence in phylogenetic analyses. The only amplicons produced by QPXF-R2 (found in only one sample) were identified as QPX, confirming the specificity of this primer pair. Among the 46 1400 bp amplicons we recovered with LabyY-PrimA, 2 sequences are related to cultivated Labyrinthulomycetes (one from muddy sediment related to *Thraustochytrium multirudimentale* and the other from sandy sediment related to *T. striatum*) and 19 are related to Aplanochytrids. 22 of the remaining 25 fell into 6 clusters (of 4, 5, 7, and 3 of 2 sequences) that appear to belong to the Labyrinthulid phylogenetic group. 2 of the remaining 3 may also be Labyrinthulids, and 1 falls into the Thraustochytrid phylogenetic group. Unfortunately, the primer LabyY is biased against several groups of Labyrinthulomycetes, particularly among the thraustochytrids. A more realistic representation of Labyrinthulomycete diversity would be produced by redesigning LabyY to be less biased and/or creating more specific primers that can target groups of Labyrinthulomycetes missed by LabyY.

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PLACEMENT OF DIVERSE AMOEBOID LINEAGES IN THE EUKARYOTIC TREE OF LIFE AND THE EVOLUTION OF 'AMOEBOZOA'

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The phylogenetic placement of amoeboid microbial eukaryotes is one of the most difficult problems to date. The paucity of comparable morphological characters has led to the lumping of many distantly related lineages into large inclusive group, such as Sarcodina, that does not reflect their evolutionary relationships. Recent analyses of molecular markers reveal members of Sarcodina are scattered in five of the six proposed supergroups. We have used multigene analyses to place six diverse amoeboid lineages - two *Nolandella* spp., *Rhizamoeba*-like sp., *Pessonella* sp., *Arcella hemisphaerica*, *Arachnula* sp. and *Trichosphaerium* sp. - in the eukaryotic tree of life. Bayesian analysis of the concatenated analysis of the four genes sequenced (SSU-rDNA, actin, alpha-tubulin and beta-tubulin) including diverse representative of eukaryotes shows that all six taxa consistently group within the 'Amoebozoa' supergroup. We further performed extensive analyses using a rate correction for the SSU-rDNA gene with increased taxonomic sampling to evaluate the resolution of

various taxonomic hypotheses. Four of our six amoeboid lineages fall within well-supported clades that are corroborated by morphology. In contrast, the placement of *Arachnula* sp. and *Trichosphaerium* sp. in the SSU genealogies are unstable as they vary by analyses. This suggests the placement of these taxa requires additional taxonomic sampling and data from slowly evolving genes. The rate corrected analyses demonstrate that SSU-rDNA has only limited signal for deep relationships within the ‘Amoebozoa’, while most of lower taxonomic hypotheses were consistently recovered. We have performed similar analyses on the enigmatic amoeba *Corallomyxa tenera* and find a close relationship between this taxon, *Gromia*, Foraminifera, and Haplosporidia (Tekle et al. in press).

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PHYLOGENY AND COMPARATIVE MORPHOLOGY OF MARINE INTERSTITIAL CERCOZOANS Chantangsi, Chitchai¹ & Leander, Brian S.²

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The diversity, morphology, and phylogeny of an emerging supergroup called the “Cercozoa” are poorly understood. Our comparative studies on the diversity of heterotrophic cercozoan flagellates sampled from different marine benthic habitats around Vancouver, British Columbia indicate that species richness in the group is far greater than we currently know. This inference is supported by a new appreciation for the morphological diversity and genetic distances between SSU rDNA sequences derived from different morphotypes. In addition, a more intensive study of the molecular phylogeny and ultrastructure of a novel uncultured cercozoan collected from marine sand has demonstrated several unusual features, including linear arrays of tiny orange bodies over the entire cell surface and several large enigmatic organelles that are reminiscent of both plastids and mitochondria. Molecular phylogenetic analyses of the SSU rDNA sequence derived from this novel cercozoan demonstrates evolutionary affinities to several environmental sequences and also a parasite of diatoms called *Pseudopirsonia mucosa*. Because members of both the Cercozoa and the Foraminifera share a synapomorphic insertion of one or two amino acid residues at the intermonomeric junction of the polyubiquitin gene, we also amplified and sequenced this gene from several uncultured cercozoans of interest, including the novel cercozoan described above, *Ebria tripartita*, *Protaspis grandis* and *Protaspis* sp. Our result showed that all of them possess one amino acid insert, namely serine, which further supports their relationships to other cercozoans.

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MORPHOLOGICAL EVOLUTION OF THE *CORALLOMYXA*, FORAMINIFERA, *GROMIA*, HAPLOSPORIDIA CLADE.

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Recent multigene molecular analyses point to a close relationship among the lineages of Foraminifera, Haplosporidia, *Gromia* and *Corallomyxa* (Tekle et al. Protist. 2007 in press). This clade, herein referred to as the CFGH clade, is also supported by similarities in the secondary structure of SSU. The emergence of this clade generated a hypothesis on the morphological evolution of pseudopodia and reticulopodia. *Gromia* is a marine testate amoeba that was once placed within foraminifera on the basis of gross test morphology. Its pseudopodia, however, are smooth and filose and differ greatly from the granular reticulopodia of Foraminifera. *Corallomyxa* is a naked amoeba that forms a reticulating network of pseudopodia more similar to Foraminifera. Members of the Haplosporidia are reduced parasites and hypothesized to have lost morphological characters of the CFGH clade. We present additional molecular and morphological data that test the CFGH hypothesis.

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MICROBIAL OBSERVATORY IN THE CARIACO BASIN - DYNAMICS OF PROTISTAN DIVERSITY ACROSS TIME, SPACE, AND CHEMICAL GRADIENTS

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The Cariaco Basin, off the coast of Venezuela, encompasses both fully oxygenated and highly sulfidic habitats, as well as the interface between the two. Our survey of 18S rRNA genes from this environment nearly doubles the rRNA gene sequence information currently available for eukaryotes in GenBank, allowing us to address two exciting and controversial questions in protistan diversity and phylogeny. The first question is: are there new “kingdom” level groups of eukaryotes waiting to be discovered? With few exceptions, claims of novel clades of the highest taxonomic order have been made in the literature on the basis of only one to a few divergent sequences, typically recovered by a single study from a single environment. As new sequences become available, these “outliers” either merge with known clades, and no longer appear “unrelated to any known taxon,” or else they merge with other new sequences as previously missed “kingdoms.” The second question is: If the present data support the presence of new “kingdom” level groups of eukaryotes, how many more should we expect to eventually discover with continued sequencing efforts? Almost every environmental rRNA survey conducted to date has claimed a substantial number of novel organisms detected, and low overlap between recovered sequences and the list of known species. Once the majority of the protists on our planet are discovered, new surveys will start “rediscovering” known organisms, especially at the higher end of the taxonomic hierarchy. Given the habitat richness of the Cariaco Basin, and how comprehensively we have surveyed it, the presence or absence of new “kingdoms” is informative on the completeness of our knowledge of global protistan diversity.

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DIVERSITY OF OLIGOTRICH AND CHOREOTRICH CILIATES IN NEARSHORE SEDIMENTS AND PLANKTON

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The diversity and biogeography of marine microbial eukaryotes remains poorly understood. We are assessing the diversity and effective population size of coastal ciliates in the orders Oligotrichia and Choreotrichia (Spirotrichea) by combining analyses of a limited number of morphospecies with culture-independent methods. We are using several morphospecies to test the hypothesis that effective population sizes of marine ciliates are large. Because of the highly amplified macronuclear genomes in spirotrich ciliates, levels of silent site variation can be assessed from multiple loci characterized from a single cell. For the culture independent work, we use oligotrich and choreotrich specific ssu-rDNA primers to explore diversity in nearshore plankton and sediments. Our preliminary analyses suggest: 1) low effective population size for some morphospecies; 2) only a few abundant species at any site; 3) the bulk of diversity is comprised of rare haplotypes; and 4) there is little overlap between sediments and plankton.

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MOLECULAR PHYLOGEOGRAPHY OF PERITRICHOUS CILIATES

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Over the past years there has been a heated debate regarding the biogeography of microorganisms. The argument has focused on microbial prokaryotic and eukaryotic organisms because of their size and abundance. There are two main schools of thought regarding this argument. On one side, researchers favour ubiquitous dispersal of protists and claim that there is no protist biogeography. On the other side, some researchers suggest that about one third of protists could be endemic. Attempts to test either of the above-mentioned hypotheses have focused on morphology and ignored the underlying potential genetic diversity. The few

molecular studies that have explored this question support both sides of the argument. Our research focuses on further investigating this area of controversy and determining whether biogeography of microorganisms is possible. A peritrichous ciliate of the genus *Carchesium* that is found in the running waters of southwestern Ontario was chosen. Fifty *Carchesium* populations were collected from the Grand River basin. The sequences of ITS1, 5.8S, and ITS2 rDNA as well as an 850bp fragment of the cytochrome oxidase subunit I were used to examine the population genetic structure. Phylogenetic analyses of the nuclear and mitochondrial sequences do not indicate a phylogeographic structure, suggesting some degree of gene flow. However, the topologies of the phylogenetic trees and the level of genetic differentiation indicate that this *Carchesium* sp. might be a cryptic species complex.

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NEW PERSPECTIVES ON THE PHYLOGENETIC RELATIONSHIPS OF SESSILID PERITRICHS: ARE MORPHOLOGICALLY 'DISTINCT' TAXA REALLY PHYLOGENETICALLY DISTINCT?

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Supposedly distinctive aspects of gross morphology in mature trophonts are still used to classify sessilid peritrichs. The unreliability of this practice has been revealed by recent molecular studies. Miao et al. (2004. *J. Eukaryot. Microbiol.*, 51:180-186) found that the sequence of the 18S ssu rRNA gene of *Vorticella microstoma*, a species with the distinctive stalk structure of its genus and family, was divergent from those of congeners and clustered with sequences of stalkless, secondarily free-swimming species (*Opisthnecta henneguyi* and *Astylozoon enriquesi*) from two other families. Although very different in gross morphology, the three species formed a well-supported clade. We tested this relationship by adding ssu rRNA sequences of two more species of *Opisthnecta*. In all phylogenetic trees, one of these clustered with *O. henneguyi* in the same clade observed by Miao et al., but the other clustered unexpectedly with stalked sessilids of the family Epistylididae in another clade. Monophyly of the *V. microstoma-Opisthnecta-Astylozoon* clade was supported by a relatively large number of shared unique nucleotide sites. These results lead to the following conclusions: 1) species of the Opisthnectidae and Astylozoidae are actually vorticellids with evolutionarily altered life cycles, 2) the 'opisthnectid' morphology has evolved convergently at least twice and is thus not a useful character for classifications or phylogenies, and 3) the Vorticellidae must be reexamined to create a valid definition of the family. Beyond sessilid peritrichs, there are many examples from all groups of eukaryotes that illustrate the danger of using gross morphology of adult stages in analyses of phylogeny and classification. Morphogenesis is always a more reliable basis for phylogeny, and molecular evidence is even more instructive. Future investigations of sessilid peritrichs must rely more on those sources of evidence.

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THE GENUS *CHONDRIA* (RHODOMELACEAE, CERAMIALES) IN THE GULF OF MEXICO

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The genus *Chondria* C. Agardh 1817 with type species *C. capillaris* (Hudson) Wynne 1991 described from Kent, England, has been reported to include close to 50 species with a tropical and temperate distribution. Falkenberg (1901) defined three subgenera in the genus: *Euchondria* for taxa with an acute apex and cylindrical thallus, *Platycondria* for taxa with an acute apex and flattened thallus, and *Coelochondria* for taxa with a blunt or depressed apex and cylindrical thallus; however, this classification has been disregarded in some recent investigations. Reports are conflicting about which vegetative characters are useful to determine species concepts. Many species are defined on the basis of sexual characteristics, but fertile material is not frequently available. Whereas previous taxonomic studies on the genus have been conducted in Australia, South Korea and southern Brazil, only the latter study included some molecular data. We are conducting a systematic study on *Chondria* centered around taxa from the Gulf of Mexico. Our molecular results on the basis on *rbcL* sequence data unveil three well-defined clades supported by vegetative features, one validating the subgenus *Coelochondria* as defined by Falkenberg.

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A MORPHOLOGICAL AND MOLECULAR INVESTIGATION OF THE GENUS *BOTRYOCLADIA* (RHODOPHYTA, RHODYMENIACEAE) IN BERMUDA, WESTERN ATLANTIC

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The genus *Botryocladia* (J. Ag.) Kylin contains some of the most recognizable red algae known. When W.R. Taylor published his flora of the tropical and subtropical western Atlantic seaweeds in 1960, he listed only two species in the genus, *B. occidentalis* (Børgesen) Kylin and *B. pyriformis* (Børgesen) Kylin, and both were listed as occurring in Bermuda. Since then, a great deal of effort has been made to better understand these beautiful red algae in the western Atlantic, so that presently 11 species of “sea grapes” are found from North Carolina to Brazil (Wynne 2005). Since we began collecting in Bermuda more than two decades ago, we have amassed hundreds of specimens of *Botryocladia* that we report on here. Using *rbcL* sequence analysis and morphological markers, we have discovered new species and new records for Bermuda. Molecular analysis of a sustained growth in one of the displays at the Bermuda Aquarium of a *Botryocladia* with an “*occidentalis*” habit forced us to reassess historical vouchers attributed to that species in Bermuda.

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UNTANGLING CYANOBACTERIAL SYSTEMATICS: ERECTION OF *EMICOLYNGBYA* GEN. NOV. FROM A POLYPHYLETIC CLADE OF *LEPTOLYNGBYA*

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Leptolyngbya contains over 150 taxa making it one of the largest genera within the Oscillatoriales. Previous molecular work using 16S rDNA gene sequence data showed this group was composed of no less than three monophyletic clades, whose members tend to exhibit widely ranging ecologies. In order to characterize monophyletic lineages, the 16S-23S Internal Transcribed Spacer (ITS) region was sequenced from 12 strains currently classified within the genus *Leptolyngbya* based on morphology. In addition, 16S rDNA gene sequences were obtained, and secondary folding structures of the D1-D1' helix, Box-B and V3 helix within the ITS regions were determined to be used as diagnostic characters for further classification. Based on the 16S rDNA sequence data, five strains fell in a well defined clade with several other previously sequenced and described strains. Further, all strains in this cluster were subaerial, and produced nodule-like cells unique in the Pseudanabaenaceae. The ITS folding patterns of the D1-D1' helix, Box-B and V3 helix were compared within this clade and against other clades within *Leptolyngbya*. Secondary folding patterns contained unique morphologies that support the 16S rDNA gene sequence data. Thus, in light of the total evidence obtained through this study, we propose the reclassification of these species to a newly erected genus *Emicolyngbya*.

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ORIGIN OF THE CYANOBACTERIAL *gnd* GENE IN SECONDARY PHOTOTROPHS AND NON-PHOTOSYNTHETIC PROTISTS

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In the secondary photosynthetic eukaryotes (phototrophs), little is known about endosymbiotic and lateral gene transfer into the host nuclear genome prior to secondary endosymbiosis. One of the important markers of endosymbiotic gene transfer from chloroplast is *gnd* gene encoding 6-phosphogluconate dehydrogenase, the second enzyme in the oxidative pentose phosphate pathway. Although previous studies showed that *gnd* genes with cyanobacterial affinity are present in several non-photosynthetic protists as well as primary and secondary phototrophs, the origins of these genes remain elusive (Andersson & Roger 2002 Curr Biol). Here we show a phylogenetic analysis including novel *gnd* gene sequences from euglenophyceae flagellates (*Euglena gracilis* and the non-photosynthetic flagellate *Peranema trichophorum*), the non-photosynthetic diplomemid flagellate *Diplonema papillatum* (Excavata) and three glaucophyte species, together with those of Heterolobosea and jakobids (Excavata) obtained from database searches. The results demonstrated that the cyanobacterial *gnd*

genes from two secondary phototrophic groups Heterokontophyta and Euglenophyceae are robustly separated from those of red algae and green plants, respectively, suggesting that these genes might have been acquired independently of the secondary endosymbioses. Furthermore, the *gnd* gene in *D. papillatum* shares the eubacterial origin with cytosol-type red algal and unikont genes, while heterolobosean and jakobid *gnd* genes occupy the basal phylogenetic position with plastid-type red algal genes within the cyanobacterial gene lineage. Thus, our data suggest that the Excavata and red algae might have acquired both cyanobacterial and eubacterial *gnd* genes in the early eukaryotic evolution. As a first step toward developing stable transgenic vectors for functional analysis of these genes, we have sequenced the entire 14,128 bp of the extrachromosomal circular rDNA plasmid in the heterolobosean *Naegleria gruberi*. Fluorescence in situ hybridization revealed that the rDNA plasmids constitute a high-order cluster within the nucleus, suggesting that they are highly organized for the efficient transcription of rRNAs.

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ALGAE-DERIVED ISOPRENOID BIOSYNTHESIS PATHWAY SUGGESTING A PLASTID IN AN OYSTER PARASITE, *PERKINSUS MARINUS*

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Dinoflagellates and apicomplexans are in close sister relationship, both harbor plastids secondarily obtained from eukaryotic algae, and exploit them for, however, diverse modes of life: phototrophy in most dinoflagellates and parasitism in apicomplexans. Their history of plastid acquisition, therefore, has been an important subject on eukaryote evolution, raising the ‘chromalveolates’ hypothesis for example. However, we have still little knowledge of basal organisms on dinoflagellates and apicomplexans lineages, which is inevitably required for discussing the course of evolution in detail. *Perkinsus marinus* (Mackin *et al.*, 1950), the notorious parasite causing mass mortalities on eastern oyster, is one of such basal organisms, of which plastids have been expected recently. In this study, we searched *P. marinus* for methylerythritol phosphate (MEP) pathway genes as molecular markers for plastids, which are responsible for *de novo* isoprenoid synthesis in plastids, and performed some sequence-based analyses. We determined the full-length sequences for six out of seven MEP pathway genes. Phylogenetic analyses revealed the obtained sequences were closely related to known orthologs of plastid-bearing eukaryotes. Possible bipartite targeting peptides, which were a characteristic feature of proteins targeted to secondarily obtained plastids, were estimated by sliding-window iteration of TargetP prediction, showing dinoflagellate affinity. These results strongly suggest that *P. marinus* harbors an ancestral organelle, or its vestigium, of dinoflagellate plastids, and the MEP pathway functions there. Additionally, observed mosaic pattern of eukaryotic MEP pathway genes suggesting complex evolutionary history of secondary plastids were discussed.

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PRELIMINARY STUDY OF DIFFERENTIAL GENE EXPRESSION DURING GROWTH AND TOXIN PRODUCTION IN *PRYMNESIUM PARVUM* (HAPTOPHYTA)

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Harmful algal blooms (HABs) of *P. parvum* produce two potent ichthyotoxins known as “prymnesins”. The chemical structures of prymnesins have been elucidated, but their biosynthetic pathways are unknown. More significantly, the genetic basis for bloom formation itself (of any HAB species) is not yet known. In this context, DNA microarrays were printed with 70-mer oligonucleotides corresponding to ~3,500 unique genes that represent a broad spectrum of functional classes and metabolic pathways. The arrays provide tools for investigating global gene expression during different phases of bloom formation, and in toxic vs. non-toxic states. Cells from axenic cultures were harvested at different phases of the growth curve to emulate different stages of the bloom cycle. Other cultures were grown in either full-phosphate or under phosphate-limiting conditions, to generate low- and high-toxicity cultures, respectively. Total cellular RNA purified from these cultures was amplified, fluorescently-labeled and hybridized to the microarrays. Pair-wise labeling of arrays with differently-labeled RNA from cells at different growth phases indicates which genes are up- or down-regulated during growth and senescence of the cultures. Similarly, simultaneous labeling of arrays with RNA

from low- vs. high-toxicity cultures reveals which genes are differentially expressed during phosphate limitation, identifying candidate genes potentially involved in toxin biosynthesis. A list of candidate genes targeted for verification and further investigation is being compiled. These studies are providing fundamental information about the metabolic and toxic status of this alga during HAB formation and decline, which could be useful for developing mitigation strategies. Also, determining gene expression patterns in these samples may provide molecular “fingerprints” for the identification and characterization of different bloom stages.

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THE CONSEQUENCES OF GENOME REDUCTION IN EUKARYOTES INFERRED FROM NUCLEOMORPH COMPARATIVE GENOMICS

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The cryptophytes are a poorly studied group of unicellular aquatic algae that acquired photosynthesis through secondary endosymbiosis, a process that occurs when a phagotrophic eukaryote engulfs a photosynthetic eukaryote and retains its plastid. Cryptophytes are unusual in that they retain the red-algal derived nucleus of their eukaryotic endosymbiont in a miniaturized form: the nucleomorph. Here we present the complete nucleomorph genome sequence of the cryptophyte *Hemiselmis andersenii* nom. prov. CCMP644. The genome is 571,876 bp in size and is comprised of three similarly sized chromosomes (~207, 184 and 179 Kbp). With the goal of better understanding the process of genome reduction and compaction in eukaryotes, we have compared the structure and coding capacity of the *H. andersenii* sequence to the only other cryptophyte nucleomorph genome sequenced thus far, the ~551 Kbp genome of *Guillardia theta*. The overall structure of the two genomes is quite similar, although sub-telomeric ribosomal DNA operons are notably absent from both ends of chromosome II and one end of chromosome III in *H. andersenii*. Spliceosomal introns (and genes encoding spliceosomal proteins) have been completely lost in *H. andersenii*. Comparison of the sizes of nucleomorph-encoded proteins in *H. andersenii* and *G. theta* to one another and to homologs encoded in unreduced genomes indicate that (1) most *G. theta* proteins are smaller than their counterparts in *H. andersenii* and (2) nucleomorph proteins are smaller than their homologs in red and green algal genomes, indicating that genome reduction can affect both coding and non-coding DNA.

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COMPLETE SEQUENCE AND ANALYSIS OF THE MITOCHONDRIAL GENOME OF *HEMISELMIS ANDERSENII* CCMP644 (CRYPTOPHYCEAE)

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Cryptomonads are a group of algae whose plastids (except for the single plastidless genus *Goniomonas*) were acquired by secondary endosymbiosis. To gain insight into cryptomonad evolution, we have sequenced the complete mitochondrial genome of *Hemiselmis andersenii* nom. prov. CCMP644 (Cryptophyceae). The *H. andersenii* circular mitochondrial genome is ~60,000 bp long and encodes 2 rRNAs, 28 tRNAs, and 39 protein coding genes. While mitochondrial gene content is quite similar between *H. andersenii* and the cryptomonad *Rhodomonas salina*, a significant amount of gene rearrangement has occurred since their divergence. The *H. andersenii* mitochondrial DNA features a large complex repeat region, which spans 32% (~20,000 bp) of the genome, and is more than four times the size of that of *R. salina*. Another unique genomic feature of the *H. andersenii* mitochondrial DNA is its lack of inverted repeats, unlike most other mitochondrial DNA with repeat regions. Molecular phylogenies of mitochondrial gene sequences will also be presented.

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PHYLOGENY OF THE EUGLENOID LORICATE GENERA *TRACHELOMONAS* AND *STROMBOMONAS* (EUGLENOPHYTA) INFERRED FROM NUCLEAR SSU and LSU rDNA

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Previous studies using the nuclear SSU rDNA and partial LSU rDNA have demonstrated that the euglenoid loricate taxa form a monophyletic clade within the photosynthetic euglenoid lineage. It was unclear, however, whether the loricate genera *Trachelomonas* and *Strombomonas* were monophyletic. In order to determine the relationships among the loricate taxa, SSU and LSU nuclear rDNA sequences were obtained for eight *Strombomonas* and twenty-five *Trachelomonas* strains and combined in a multigene phylogenetic analysis. Conserved regions of the aligned dataset were used to generate maximum-likelihood and Bayesian phylogenies. Both methods recovered a strongly supported monophyletic loricate clade with *Strombomonas* and *Trachelomonas* species separated into two sister clades. Taxa in the genus *Strombomonas* sorted into three subclades. Within the genus *Trachelomonas*, five strongly supported subclades were recovered in all analyses. Key morphological features could be attributed to each of the subclades, with the major separation being that all of the spine-bearing taxa were located in two sister subclades while the more rounded, spineless taxa formed the remaining three clades. The separation of genera and subclades were supported by 42 distinct molecular signatures (33 in *Trachelomonas* and 9 in *Strombomonas*). The morphological and molecular data supported the retention of *Trachelomonas* and *Strombomonas* as separate loricate genera.

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PHYLOGEOGRAPHY, MORPHOLOGICAL VARIATION AND TAXONOMY OF THE TOXIC DINOFLAGELLATE *GAMBIERDISCUS TOXICUS* (DINOPHYCEAE)

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Gambierdiscus toxicus Adachi et Fukuyo 1979 is a toxin-producing marine dinoflagellate responsible for the syndrome known as ciguatera, which sickens thousands of people every year. Little is known regarding the global genetic structure of this species; therefore, it is unclear whether documented variation in toxin production and outbreaks of ciguatera are a result of ecological triggers or the presence of different genetic strains across the geographic range of *G. toxicus*. We examined the molecular phylogeny and morphological characteristics of globally distributed *G. toxicus* isolates by sequencing part of the large subunit (LSU) and small subunit (SSU) ribosomal DNA and examining the thecal architecture using scanning electron microscopy (SEM). Our analyses showed that *G. toxicus* is comprised of at least four distinct lineages separated by substantial genetic distances. One of the clades is morphologically distinct; however, the remaining three lineages are morphologically homogeneous and may represent cryptic species. The association between phylogenetic relationships and phenotypic characteristics in dinoflagellates is often tenuous; evaluating morphological characteristics within a phylogenetic framework is, however, an effective method of distinguishing phenotypically plastic from stable characteristics and for the identification of ancestral versus derived features. Our SEM analyses showed that an important morphological feature used to distinguish *Gambierdiscus* morphospecies was variable among isolates of *G. toxicus* and did not reflect phylogenetic groupings; hence its utility as a diagnostic feature is questionable. Our molecular and morphological analyses further suggest that a taxonomic revision to this genus is appropriate.

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ASSESSING DNA BARCODES AS AN IDENTIFICATION TOOL IN DINOFLAGELLATES

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Dinoflagellates, are a diverse protistan group of approximately 3000 known species but are fairly poorly catalogued especially in benthic and non-cultivable species. We aimed to test whether DNA barcodes can really differentiate between dinoflagellate species and strains and therefore be used as an effective tool to measure biodiversity. We collected 77 different dinoflagellate species from several major algal culture

collections and obtained 150 barcode sequences from DNA markers, the cytochrome oxidase I (COI) gene and the nuclear internal transcribed spacer sequence (ITS). Comparing inter-generic, uncorrected pairwise distances for COI, we could distinguish between genera and species in most cases, which grouped together according to accepted phylogenetic relationships. However, for the case of the toxic *Alexandrium*, a notoriously difficult species to differentiate morphologically, we found virtually no genetic distances between different species, a problematic issue given that a DNA barcode should uniquely identify each species. We then used ITS as an alternative barcode and found that it provided excellent species differentiation in those samples, using the same methods (165 fold increase in pairwise distances in *Alexandrium* species compared to those of COI) and therefore could be used as a second barcode in certain species. However, the use of an ITS barcode for dinoflagellates may have practical difficulties as we found ITS sequences of a significant proportion of species too divergent to be aligned between genera, plus there is evidence of paralogy.

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CHARACTER EVOLUTION IN DINOFLAGELLATES WITH COMPLEX ORGANELLES

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This talk will address the molecular phylogeny and evolutionary morphology of polykrikoids and warnowiids. Both polykrikoids and warnowiids possess several distinctive organelles, such as complex nematocysts. Moreover, all warnowiids possess a complex eye-like organelle called the ‘ocelloid’, and some species (i.e. *Erythrospidinium*) have also developed a dynamic appendage called the ‘piston’. Phylogenetic analyses of small subunit (SSU) rDNA sequences derived from manually isolated, uncultured cells of *Phaeopolykrikos beauchampii*, *Polykrikos kofoidii*, *Polykrikos lebourae*, *Polykrikos herdmanae*, *Proterothropsis* sp., *Nematodinium* sp. and *Warnowia* sp. enabled us to test and refine a hypothetical framework built from comparative morphology of these species. A well supported *Polykrikos* clade formed the nearest sister lineage to *Gymnodinium fuscum*, the type species of the genus. These results demonstrated that pseudocolonies in polykrikoid dinoflagellates evolved at least two times independently, and the best synapomorphy for a *Polykrikos* clade was the presence of two nuclei irrespective of zooid number. Sequences from the three species of warnowiids formed a well-supported clade within the *Gymnodinium sensu stricto* clade (including *Polykrikos*, *Phaeopolykrikos* and *G. fuscum*). These phylogenetic data not only provided new insights, but also new uncertainties about the evolutionary history of this group of athecate dinoflagellates.

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A TAXONOMIC STUDY OF A *PROTOPERIDINIUM OBLONGUM*-COMPLEX AND ESTABLISHMENT OF CULTURES OF *PROTOPERIDINIUM* WITH NON-CELLULAR FOOD ITEMS

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The genus *Protoperidinium* is a marine thecae heterotrophic dinoflagellate assemblage and known as having a variety of cyst types. Although several morphologically different cells were observed in both the motile cells and cysts of ‘*Protoperidinium oblongum*’, it is not clear their mutual relationships and whether these differences represent species difference or intraspecific variety. We undertook cyst-incubation experiments and investigated two types of cysts, both of which have been identified as cysts of *P. oblongum*. We also studied morphology of germinated motile cells and then these cells were subjected to single-cell PCR and the SSU and the part of LSU rDNA sequences were determined. As a result, two types of cysts produced genetically different motile cells and they could be distinguished by thecal plate arrangement. After taxonomic consideration, we realized that these two types of motile cells are different from true *P. oblongum*. For one species, we proposed to raise it to specific rank, making a new combination *Protoperidinium inaequale* comb. et stat. nov. (Basionym: *Peridinium oblongum* var. *inaequale*). One other species seems to resemble *Peridinium oblongum* var. *symmetricum*, but the taxonomic proposal to raise this variety to specific rank was reserved. This was because there is another species which possesses similar morphology, but genetically distinct from our species and we were not able to decide which is more closely related to *P. oblongum* var. *symmetricum*. For search for non-cellular food items for growing *Protoperidinium* species, we fed the dinoflagellates with many different kinds of organic matters. Finally, we were able to establish cultures of *P.*

crassipes by feeding them with rice flour. In addition to the above topic, here we report the results of our feeding experiments briefly.

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VARIABLE ABUNDANCE OF *KARLODINIUM VENEFICUM* IN US EAST COAST AS DETECTED BY A DUAL-GENE REAL-TIME PCR ASSAY

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Karodinium veneficum is an ichthyotoxic dinoflagellate and suspected to be responsible for massive fish kills. Because it is morphologically similar to *Pfiesteria* and related dinoflagellates, accurate abundance data of *K. veneficum* in the natural environment is essentially lacking. In this study, a Real-Time PCR assay was developed using ferredoxin (158bp; KvFERR) and a rDNA locus containing partial ITS1, 5.8S, and partialITS2 (386bp; KvITS), and geographic and temporal distribution of *K. veneficum* was investigated. Primers designed for both genes were shown to be sensitive and *K. veneficum*-specific in general, although ITS exhibited 10-fold higher sensitivity and rare low-level non-specific PCR amplification occurred for ferredoxin. Both genes generally gave similar quantitative results. *K. veneficum* was detected in Harbor of Clipper II on Pelican Island in Texas, Neuse River in North Carolina, Chesapeake Bay in Maryland, Long Island Sound in New York and Connecticut, Narragansett Bay in Rhode Island, Boston Harbor in Massachusetts, Rockland Harbor and Trenton Townline in Maine. Frequency of occurrence and abundance of *K. veneficum* were highest in Neuse River (70.8% of the 130 samples during July-2002 to July-2003 with the highest concentration of 1.7×10^3 cells mL⁻¹) and Chesapeake Bay samples (52.9% of the 17 samples in September-October 2002 with the highest concentration of 26 cells mL⁻¹), than other positive locations (<12 cells mL⁻¹). Strikingly, the ITS sequences from the 22 selected positive samples from different geographic locations were identical, suggesting that the *K. veneficum* populations in this wide geographic range (Texas to Maine) was genetically homogenous. However, extensive third codon position substitution in ferredoxin was detected for the 25 selected positive samples, indicating gene duplication in this species. We conclude that 1) the KvITS-KvFERR Real-Time PCR assay is sensitive and specific for detecting and quantifying *K. veneficum* in the natural environment; 2) *K. veneficum* abundance was substantially higher than *Pfiesteria* spp. in the high fish kill estuaries (Neuse River, Chesapeake Bay).

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NATURAL VS. ANTHROPOGENIC NITROGEN UPTAKE IN *ULVA* AND *GRACILARIA*, TWO BLOOM-FORMING MACROALGAE

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Macroalgal blooms are becoming increasingly common ecological formations in shallow bays and estuaries worldwide. These blooms are spatially and temporally variable and are the result of rapid, seemingly uncontrolled growth of one to several species of macroalgae. They may negatively impact other shallow marine habitats such as seagrass and shellfish beds and are frequently regarded as nuisance species. In Narragansett Bay (RI), several species of green algae (primarily *Ulva*) and red algae (primarily *Gracilaria*) form dense aggregations during the summertime. Nitrogen is one of the primary limiting nutrients for macroalgae, and the formation of these blooms is frequently attributed to increased inputs of anthropogenic sources of nitrogen. In this study, we investigated the relative contributions of natural and anthropogenic (from sewage) nitrogen to macroalgal growth via stable nitrogen isotope ratios. We cultured *Ulva* and *Gracilaria* in water collected from four locations in Narragansett Bay along a latitudinal gradient of anthropogenic nitrogen inputs and examined the nitrogen isotopic signature as $\delta^{15}\text{N}$. Our northernmost site was at the head of the bay near a major urban center, Providence, while our southernmost site was near the mouth of the bay in a more rural area. We found significant differences in the $\delta^{15}\text{N}$ signature of algae cultured among these different water sources; algae grown in our northern water treatments had enriched signatures of $\delta^{15}\text{N}$ (and thus more anthropogenically derived nitrogen) relative to our southern treatments. These data, when coupled with a previous general model of circulation patterns, will permit the rapid estimation of the relative contribution of the major nitrogen sources to this bay, and their impacts on the population dynamics of local organisms.

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PALATABILITY OF *PALMARIA DECIPIENS* AND ITS ENDO/EPIPHYTE *ELACHISTA ANTARCTICA* TO THREE COMMON ANTARCTIC AMPHIPODS

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Although many Antarctic macroalgae are known to produce secondary metabolites that aid in their protection against grazing, it is suspected that the rhodophyte *Palmaria decipiens* is often grazed upon by primary consumers, especially amphipods and fish. *P. decipiens* is host to a number of epiphytes including the endo/epiphyte, *Elachista antarctica*. *E. antarctica* is a filamentous phaeophyte only found growing within, and emerging out of the thallus of *P. decipiens*. It is surprising *E. antarctica* is present only on a palatable species of macroalgae considering that the standing biomass of other, unpalatable species is very high. In order to confirm the palatability of *P. decipiens*, and begin to understand the relationship between *E. antarctica* and *P. decipiens*, feeding assays were conducted with three amphipods commonly associated with *P. decipiens*: *Prostebbingia gracilis*, *Gondogeneia antarctica*, and *Oradarea bidentata*. Feeding assays were conducted to determine consumption rates (mg algae mg amphipod⁻¹ h⁻¹) for all three amphipod species. In feeding assays, one amphipod was isolated with either *P. decipiens* or *E. antarctica* for set time. Preference experiments where an individual amphipod was given a feeding choice between *P. decipiens* and *E. antarctica* were also conducted using all three amphipod species. *P. gracilis* did not consume *P. decipiens* but did eat *E. antarctica*. *G. antarctica* consumed both species but ate *P. decipiens* at a significantly faster rate. *O. bidentata* fed on *E. antarctica* at a significantly faster rate than *P. decipiens*.

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DISCOVERY OF SECONDARY CELL WALLS AND LIGNIN PRECURSORS IN THE JOINTS OF THE ARTICULATED CORALLINE ALGA *CALLIARTHON*

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Joints (genicula) in the wave-swept articulated coralline *Calliarthron* (Rhodophyta, Corallinaceae) lend flexibility to calcified fronds, helping them resist forces imposed by breaking waves. Genicular tissue is stronger and stiffer than other algal tissues. Previous studies demonstrated that tissue strength results from thickened genicular cell walls, while tissue stiffness is likely a consequence of distinct material composition. Stiff, thickened cell walls are characteristic of the vessel and fiber elements of terrestrial plant xylem, which produce secondary cell walls fortified with lignin. Transmission electron micrographs revealed the presence of secondary cell walls in genicular cells that develop after cell elongation ceases. Mass spectrum analyses demonstrated that *Calliarthron* genicula contain three distinct monolignols, which polymerize to form P-, G-, and S-lignins in terrestrial plants. Secondary cell walls and monolignols are known only from terrestrial plant tissues and have never been described in marine algae. Lignin histochemistry and lignin-specific antibodies corroborated mass spectra results, suggesting that G-monolignols are concentrated in secondary cell walls, while P-, G- and S-monolignols may be present at lower levels in primary walls. Data presented here suggest the need to re-examine the evolutionary history of lignified cell walls. Developmental pathways for both secondary cell walls and monolignols may have evolved in a common ancestor of red and green algae more than 1 billion years ago or may have evolved convergently in coralline algae and land plants as adaptations to mechanical stress.

205**SEAWEED BIODIVERSITY OF THE GULF OF CALIFORNIA ESTIMATED FROM A CENTURY OF HISTORICAL RECORDS**

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A variety of diversity indices has been used to express the complexity of species biodiversity in a particular environment. Most indices (Simpson being a notable exception) are sensitive to the degree of sampling effort and, therefore, highly sensitive to sample size and non-comparable across studies. When the purpose is to estimate the biodiversity of large coastal environments, it is practically impossible to obtain exhaustive census with equal sampling effort to estimate these indices. Recently, Clark and Warwick (1998, 2001) have proposed the use of taxonomic distinctness indices; average taxonomic distinctness ($\Delta+$) and variation in taxonomic distinctness ($\Lambda+$), in comparing historic data sets and studies for which sampling effort is uncontrolled, unknown or unequal due to their lack of dependence of its mean value on sampling effort. These indices offer also the possibility to be tested for departure from the expected value. So, localities that have attracted differing degrees of sampling effort are potentially directly comparable with $\Delta+$ for the full inventory. In this study we compiled an historical record of approximately one century of seaweed literature (1911 to 2005) from the Gulf of California, considered one of the most pristine marine environments in the world, to estimate the biodiversity ($\Delta+$ and $\Lambda+$) of the gulf as a whole and compared among its different regions. The possibility to estimate biodiversity from historical presence/absence data provides the opportunity not only to establish a base line for biodiversity on large scale environments with relatively low effort, but to infer changes from when these environments were pristine to present.

206**ALGAL CONTRIBUTIONS TO THE ANCIENT ORGANIC CARBON CYCLE**

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We report new results of chemical and phylogenetic analysis of lipid biomarkers in modern organisms that suggest the traditional assumption that green algal phytoplankton were the dominant primary producers in ancient oceans may be inadequate to explain the record of preserved ancient organic matter. The role of green algal phytoplankton in past ecosystems may have been overstated, and green macrophytes as well as other early divergent lineages of the Plantae may have been larger contributors to ancient preserved organic matter than has traditionally been assumed. Sterols, eukaryotic lipids that are commonly used as molecular fossils, may be the most robust line of evidence for elucidating eukaryotic diversity and the source of preserved organic matter in ancient sediments. They also provide a fossil record for organisms that may not otherwise leave a fossil record. It is well established that there is a rapid rise to dominance of dinoflagellates, coccolithophores, and diatoms, in the Mesozoic, and it is believed that these modern phytoplankton groups replace green algal phytoplankton dominated seas. The strongest evidence for this turnover is the ratio of C28/C29 sterols through time, and the interpretation of this biomarker evidence relies on a strong correlation between green algal phytoplankton and C29 sterols. We conducted a quantitative analysis of sterol profiles for all major groups in the Plantae (Red and Green algae and the Glaucocystophytes) to investigate the hypothesis that C29 sterols, the dominant sterols in the Neoproterozoic and Paleozoic, were sourced by Green Algae. Data from 182 taxa was analyzed for significant taxonomic, phylogenetic and ecological trends. Our results highlight an evolutionary trend in sterol production that could impact interpretations of C29 sterols fossil record, and gives some support for the current hypothesis that green algal phytoplankton may have been ecologically important in pre-Mesozoic oceans. However, this analysis also offers an alternative hypothesis for the origin of the most ancient preserved algal organic matter that suggests macroalgae may have played a larger role in the global carbon cycle than has previously been considered.

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DEVELOPMENT OF A REAL-TIME QUANTITATIVE PCR ASSAY FOR THE THRAUSTOCHYTRID QPX (LABYRINTHULOMYCOTA)

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QPX (Quahog Parasite Unknown), a thraustochytrid parasite of the hard clam *Mercenaria mercenaria*, has caused significant mortality of hard clam stocks in many locations along the east coast of the United States and Maritime Provinces of Canada since the 1960's, including an economically devastating outbreak in Raritan Bay NY during the summer of 2002. We set out to develop a SYBR Green-based real-time quantitative PCR (QPCR) assay that will allow us to detect and enumerate QPX both in clams and in environmental samples. Existing 18S rDNA-targeted primers specific for QPX proved unsuitable for QPCR. Instead, our previous work on the variability of rRNA operon sequences among several QPX isolates revealed that the ITS region (including ITS1, the 5.8S rRNA gene, and ITS2) offered alternative primer targets. We designed and tested nine primer pairs targeted to the ITS region. Three of the primer pairs proved artifact-free and specific for QPX in several regular PCR tests. When tested in the QPCR format, one of these primer pairs clearly had the best amplification efficiency, detection limit, and detection range. We estimate that the QPX genome contains approximately 200 copies of the rRNA operon, giving the assay a theoretical detection limit well below 1 cell per reaction. We routinely detect the presence of QPX cells in clams that are histologically QPX-negative, demonstrating the greater sensitivity of the QPCR approach. We have also detected QPX in some sediment samples, but never in samples of particulate matter collected from the overlying water column. The greatest technical challenges to improving the technique are in the efficiency of extracting QPX DNA from various sample types and in overcoming PCR inhibition by co-purified substances.

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REEVALUATION OF THE ROLE OF NAKED, AMOEBOID PROTISTS IN BACTIVORY AND MICROBIAL CARBON FLUX IN COASTAL MARINE AND ESTUARINE WATERS

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Recent observations in several coastal marine systems have revealed that naked amoeboid protists (amebas) are much more abundant in the plankton than previously thought, reaching 10^4 - 10^5 per liter. These numbers suggest that amebas may be important bacterivores in many systems. However, their variable morphology has previously defied estimation of biovolume. Using a newly-established relationship between cell size measurements and ameba biovolume, their carbon content, and that of other bacterivorous protists, was estimated during a pilot study in the Hudson River estuary. Amebas comprised 2-22% of the total planktonic bacterivore carbon; less than nanoflagellates but comparable to the ciliates, a protist group that has received much more attention. Mean specific ingestion and clearance rates on bacteria, and ameba growth efficiency (the first estimates of these parameters under natural conditions) showed that amebas can be major bacterivores in the estuary, particularly in summer, and therefore play an important role in carbon transfer through the food web.

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EFFECTS OF TEMPERATURE, IRON AND CO₂ ON MICROZOOPLANKTON ASSEMBLAGES IN THE ROSS SEA, ANTARCTICA AND THE NORTH ATLANTIC SPRING BLOOM

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The Ross Sea, Antarctica and North Atlantic are both sites of large-scale annual phytoplankton blooms that shift spatially and temporally between diatoms and phytoflagellates. These regions have been predicted to

experience large physical and chemical changes in future climate scenarios. However, little is understood about the potential response of phytoplankton assemblages to this changing climate. Even less is known about potential community dynamics within microzooplankton assemblages. Shipboard experiments were conducted with whole plankton assemblages from each region, examining the effects of temperature, iron and CO₂ on plankton ecology and nutrient cycling. Increasing temperature and iron individually and in concert increased phytoplankton abundance and nutrient drawdown in the Ross Sea experiment. Increasing temperature alone resulted in increased total microzooplankton abundance without large effects on microzooplankton community composition. However, increasing iron concentrations led to a decrease in total microzooplankton abundance and a large shift in microzooplankton community composition. Increasing temperature and CO₂ individually and in concert resulted in increased phytoplankton abundance and nutrient drawdown in the North Atlantic experiment. Increasing temperature alone resulted in minimal changes in microzooplankton abundance but huge shifts in community composition. Increasing temperature and CO₂ together resulted in initially rapid growth of microzooplankton, but the assemblage crashed mid-experiment in concert with increasing abundance of a potentially noxious prymnesiophyte. We believe that the effects of these chemical and physical factors on the microzooplankton assemblage were caused by a combination of direct effects on microzooplankton physiology as well as indirect effects due to changes in phytoplankton community composition. These results have important implications for top-down controls on phytoplankton assemblages in a future greenhouse world.

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DENSITY, CARBON CONTENT, AND POTENTIAL ATMOSPHERIC YIELD OF RESPIRATORY CARBON DIOXIDE OF NON-TESTATE AMOEBAE IN A TERRESTRIAL BRYOPHYTE COMMUNITY: ECOLOGICAL AND BIOGEOCHEMICAL IMPLICATIONS

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Terrestrial bryophytes (mosses) produce extensive patches of growth, especially at high latitudes where they form an almost continuous ground cover in black spruce forests, fix more atmospheric carbon than the trees, and are currently a net sink for CO₂. With increasing global warming, however, respiratory release of CO₂ by moss-associated microbes may exceed the amount fixed by the moss, contributing to an elevated greenhouse effect. Yet, little is known about the eukaryotic microbial communities of mosses, especially the non-testate amoebae (gymnamoebae). The gymnamoeba density (number/g moss dry weight), carbon content (μg/g moss dry weight) and potential respiratory production of CO₂ were assessed over several seasons in a thick growth of the moss *Hypnum* sp. at Torrey Cliff Reserve, N. Y. The amoeba carbon content ranged from 0.22 to 5.3 μg/g moss dry weight and was highest in spring and autumn. In January 2006, amoebae carbon was assessed in freshly collected moss samples and in a laboratory incubated sample from the same site kept at 20 °C for 1 week, simulating a winter thaw. The amoeba carbon content increased from 1.9 to 5.0 μg/g moss dry weight, which was similar to the amount (5.3 μg/g moss dry weight) in a natural “thaw” in January 2007. Gymnamoeba carbon content ranged as high as ~ 25% of the carbon content of the moss-dwelling prey bacteria. Thus, it may be a significant source of carbon in food webs and is a potential reservoir that can be released as atmospheric respiratory CO₂ in addition to yields from the bacteria that are most typically cited as a terrestrial biotic source of elevated atmospheric CO₂.

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USING PROTISTS TO TEACH EVOLUTION

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The recent resurgence of creationism in the form of the Intelligent Design (ID) movement has placed increasing pressure on science educators at all levels (K-College). An understanding of protists offers a unique opportunity to teach several important aspects of evolutionary theory by drawing upon examples of protistan biology. Concepts such as the origin of new species, increases in genetic complexity, and the emergence of complex biological structures can all be easily illustrated using protistan models. In particular the creation of new protistan species via symbiosis, the origin and importance of plastids, and the emergence of the eukaryotic cilium are all examples of complex biological systems that can be used to refute objections to speciation, new

genetic information, and the falsity of irreducible complexity (*sensu* Behe). Examples of these, and other model systems for explaining evolutionary concepts will be discussed.

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