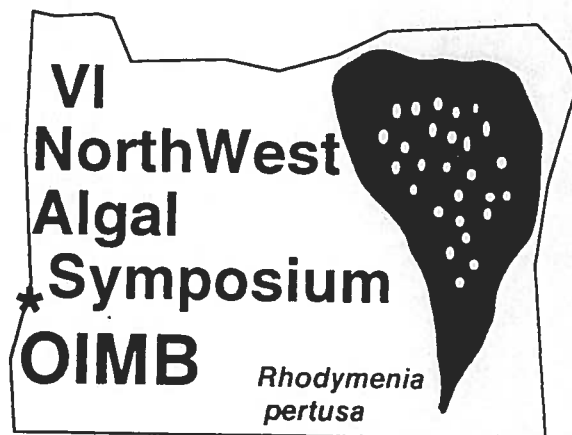


The VI Annual Northwest Algal Symposium will be held at the Oregon Institute of Marine Biology (OIMB), Charleston, Oregon, 503-888-2581. The local organizer is Michelle Wood (Dept. of Biology, University of Oregon, Eugene, Oregon 97403; OMNET: M.Wood; Tel. 503-346-2862, FAX 503-346-2348) and the scientific program is being coordinated by Eric Henry (Oregon State University, Dept. of Botany and Plant Pathology, Oregon State University, Corvallis, Oregon 97331-2902, Tel. 503-737-5271; FAX:737-3045) and Michelle Wood.



LODGING AND MEALS

ON SITE HOUSING - The Oregon Institute of Marine Biology has dorm spaces for 50 people, the majority of these are beds in open loft areas housing 8 - 18 people. A few individual rooms are available. The dining hall can provide meals for 100 people. You will need to bring your own bedding (sleeping bag), pillow and towels.

MOTELS - Please make your own reservations directly with motels.

A block of rooms has been reserved for the Nwas at Captain Johns Motel, Charleston which is a 2 minute walk to OIMB. Mention the Northwest Algal Symposium when you make your reservation.

Captain John's Motel, Charleston, Phone: 503-888-4041, a 2 minute walk to OIMB.
Rates: \$35-50 (per room, double or single occupancy)

Edgewater Inn, Coos Bay, Phone: 503-267-4023, 8 miles from OIMB
Rates: \$46-56 + tax (double occupancy)

CAMPING - Camping is available approximately two miles from OIMB at Bastendorf County Park. Water, fire ring, and electrical hook-up are available at all sites. The fee is \$5.00 per night without hook-up and \$7.00 with hook-up. Reservations are not accepted. The park telephone number is 503-888-5353. To reach the park follow the Cape Arago Highway south from Charleston.

TRANSPORTATION

The location of OIMB is indicated on the attached map. North Bend airport is 7 miles from OIMB. It is served by Horizon Air (a partner with Alaska Air) via Portland or Eugene. We will meet all the incoming flights to the North Bend airport on Friday, March 20 and provide rides to the airport Sunday afternoon. If you arrive at other times, the taxi fare to OIMB is about \$10. Approximate driving times to Charleston: Vancouver - 12-13 hours, San Francisco - 11 hours, Seattle - 8 hours, Portland - 5 hours, Corvallis - 3 1/2 hours, Eugene, 2 1/2 hours.

PROGRAM - VI NORTHWEST ALGAL SYMPOSIUM

Friday, March 20

- 1:00 - 6:00 arrival, check-in, and putting up posters (Dining Hall)
- 2:30 Field trips leave OIMB from dining hall
- 6:00 Dinner, Welcome Messages, and Memorial Tribute to Harry Phinney
- 7:30 - 10:00 - Combined social and poster session (Cash Bar)

Saturday, March 21

- 7:30 - 8:00 Breakfast
- 8:15 - 10:30 Algal Based Food Web Symposium (Boat House Auditorium)
 - Sherr, E.B. and B.F. Sherr. *Microbial food webs: herbivory on the microscale.*
 - Power, M.E. *Algal-based food webs in rivers.*
 - Menge, B.A. *Bottom-up and top-down community regulation: are rocky intertidal food webs an exception?*
- 10:30 - 10:45 Break
- 10:45 - 12:25 - Contributed Session I (Boat House Auditorium)
- 12:45 - 1:15 - Lunch
- 1:30-5:00 *Image Analysis Workshop* (Classroom Building I)
 - 1:30 - 2:30 - Lisa Phelan (Imaging Fundamentals, Inc.) *Basics of Image Analysis*
 - 2:30 - 3:00 - Bob Stout (Bartels & Stout) *Microscope-Based Image Analysis: Optimizing the Image*
 - 3:00 - 5:00 - Workshop Sessions with Jimmy Walker (Jandel, Inc.), Chad Hewitt (OIMB) & Bob Stout, and Lisa Phelan
- 5:15 Business Meeting (Boathouse Auditorium)
- 6:30 Banquet followed by poster session and social (Dining Hall; Cash Bar)
Banquet Speaker, Jane Lubchenco, *The Sustainable Biosphere Initiative*

Sunday, March 22

- 7:30 - 8:00 Breakfast
- 8:00 Collecting site guides leave OIMB from dining hall (Low tide is -0.6, 0840*)
- 9:00 - 9:30 Sleepyhead coffee and doughnuts in poster session room
- 10:00 Invited Lecture (Boathouse Auditorium)
 - Steve Fain, *New Methods for Analysis of Population Genetics in Marine Plants*
- 10:45 - 12:25 Contributed Session II (Boathouse Auditorium)
- 1:00 - 1:30 Lunch
- 1:30 Take down posters, check-out, Registrants pick up commemorative mugs, depart

*Bring your own bucket

CONTRIBUTED PAPERS - VI NORTHWEST ALGAL SYMPOSIUM

Session I (Saturday AM)

- Lucey, W.P., B. Jeffs, K. Congdon, A.P. Austin, and B. Moore. Diminished water quality downstream of remotely located, but recreationally accelerated, wilderness development: end-of-pipe vs. watershed management.
- Justesen, E.M. The reproductive life histories and morphology of *Audouinella purpurea* (Acrochaetiaceae, Rhodophyta) and *Pilayella littoralis* (Ectocarpaceae, Phaeophyta) under low light conditions.
- Belhadri, A. and G. Brugerolle. Identification by monoclonal antibodies of an intermicrotubular cement in the feeding apparatuses of the euglenoids: *Entosiphon*, *Peranema*, and *Ploeotia*.
- Lennihan, R. Water relations and acetylene reduction rates in cyanobacterial crusts in the high Arctic.
- Reuter, J. The importance of iron to open ocean phytoplankton.

Session II (Sunday AM)

- Cavalier-Smith, T. The origins of algae.
- Valentin, K. The evolution of algal plastids and their genomes.
- Reynolds, A.E., B. L. McConaughy, R.A. Cattolico. Transcriptional regulation of photosynthetic genes during a diel cycle in the chromophytic alga *O. luteus*.
- Graves, M.V. and R.H. Meints. Characterization of the major capsid protein and cloning of its gene from algal virus PBCV-1.
- Hunt, B., A. Zeller, A. Wexler, and D.F. Mandoli. Improved feasibility for developmental genetics in *Acetabularia*: less culture maintenance, shorter cell life cycle and improved mating efficiency.

In-field split-stream mesocosms: a periphyton bioassay to measure ortho-phosphate induced biomass accrual.
Austin, Alan.P., Cori Barraclough, Wm.P. Lucey.

Using a microhaptobenthic community contained within stream-troughs (split-mesocosms), a field study (1988-1991) was used to model sewage-based (from the recreational Resort Community of Whistler) eutrophication of the Cheakamus River, British Columbia. The ortho-phosphate concentration threshold required to significantly increase algal production was determined; in-stream studies² determined that periphyton biomass accrual above 2500 ug/cm would adversely affect aesthetics and possibly fisheries. The periphyton bioassay repeatedly measured an ortho-phosphate threshold, above which biomass accrual increased significantly, at approximately 0.05 ug-P/L, a value at least an order of magnitude below chemically detectable levels. The experimental and in-stream biomass accrual patterns were used to determine future maximum permissible sewage treatment plant discharges. The data suggests that continued and enhanced treated-sewage effluent discharge, coupled with recent changes in B.C. Hydro's reservoir operating procedures, would further reduce downstream water quality and habitat. Establishing the river's limited capacity to assimilate nutrients resulted in a Liquid Waste Management Plan, replacing the traditional resource planning practise of an 'end-of-the-pipe' discharge permit.

USE OF ARTIFICIAL STREAM-TROUGHS TO ASSESS PERIPHYTON RESPONSE TO NUTRIENT LOADING FROM HARVESTED FORESTS.

A. Austin, D. Grant; K. Nickolichuk, P. Lucey, S. Irwin*. University of Victoria, Victoria, B.C. Canada. *Greater Victoria Water District, 479 Island Highway, Victoria, B.C.

Logging has been practiced since the early 1900's in the Greater Victoria Water District (GVWD), the potable water supply for Victoria, B.C. Recently, in the GVWD reservoirs, taste and odour problems have been associated with algal blooms that may have resulted from elevated phosphorous concentrations originating from increased runoff from harvested hillsides. To date no research in GVWD watersheds has been conducted which links logging practices with eutrophication. The primary objective of this study was to measure the fluctuations in water quality (nutrient concentrations) of ephemeral streams draining forested and harvested hillsides. To meet this objective, two artificial stream-trough systems were established adjacent to two ephemeral creeks; one draining a recently logged area, the other forested. Because very low or "below detectable limits" of ortho-phosphorous cannot be measured by chemical analytical techniques, a periphyton bioassay was used. Treatments consisted of a series of increasing phosphorous concentrations (0.1, 0.25 and 0.5 ug/l) and controls (ambient P). Through the use of regression analysis, it will be possible to determine the ambient phosphorous concentration in the controls based upon periphyton biomass accrual as a function of increasing ortho-phosphorous concentration. A second objective of this study was to determine whether irradiance or nutrients (ortho-phosphorous) limit periphyton growth in streams with and without canopy cover. To meet this objective, additional treatments (ambient P and 0.25P+) were run under reduced irradiance to quantify irradiance vs. nutrient limitation on the growth of periphyton communities in forested and logged streams. In-stream artificial sampling surfaces and natural surfaces within the latter habitats are being routinely sampled and periphyton structure (species composition and biomass, measured as chlorophyll *a*, and dry mass) measured.

IDENTIFICATION BY MONOCLONAL ANTIBODIES OF AN INTERMICROTUBULAR CEMENT IN THE FEEDING APPARATUSES OF THE EUGLENOIDS: *ENTOSIPHON*, *PERANEMA* AND *PLOEOTIA*.

Abderrahmane Belhadri and **Guy Brugerolle**. Laboratoire de zoologie-Protistologie, Université Blaise Pascal Clermont-Ferrand 63177 Aubiere Cedex, France.

Monoclonal antibodies (MAbs) were produced against cytoskeleton of the phagotrophic euglenoid cell *Entosiphon*. One of these, the MAb IID12, labelled the feeding apparatus or siphon by immunofluorescence and by immunogold staining. The intermicrotubular cement abundant in the upper part of the siphon was decorated. When applied to *Peranema* and *Ploeotia* (two others phagotrophic euglenoids), this MAb decorated the same material or cement present in their feeding apparatuses. Immunoblotting after SDS-PAGE revealed that the epitope recognized by the MAb IID12 is localized in two polypeptides of 58-66 kD in *Entosiphon*, 82-84 kD in *Peranema* and 56-60 kD in *Ploeotia*. Two dimensional electrophoresis of *Entosiphon* skeleton showed three spots, with pI ranging between 6 and 7, recognized by the MAb.

This antibody was used in combination with an anti-tubulin MAb to follow different stages of siphon morphogenesis during the division of *Entosiphon* cells. Moreover an electron microscopic study based on serial sections gave new information on siphon construction and positioning. When the parental siphon breaks and disappears, the cement depolymerizes first liberating the microtubules which disorganize and disassemble afterwards. Conversely the cement seemed to assemble tardily in the two newly formed siphons and probably serves to stabilize the newly formed structures.

THE ORIGINS OF ALGAE

T. Cavalier-Smith. Canadian Institute for Advanced Research Evolutionary Biology Program, Department of Botany, University of British Columbia, Vancouver, B.C., Canada, V6T 1Z4

One can divide algae into proalgae (cyanobacteria and their probably polyphyletic prochlorophyte derivatives), eualgae (those that evolved from a biflagellate protozoan by the conversion of an endosymbiotic cyanobacterium into a chloroplast), and metaalgae (those that evolved by incorporation of a eualga into a protozoan). I shall argue that chloroplasts and eualgae evolved once only and that green algae, red algae, and glaucophytes all diverged from the ancestral eualga. The status of photosynthetic dinoflagellates and euglenoids, which both have three membranes in their chloroplast envelope, is less clear, but I think it most likely that even they diverged directly from the ancestral eualga. The Chromista (cryptomonads and chromophyte algae) are clearly metaalgae, but whether they are mono or biphyetic is still debatable. Chlorarachnion is a metaalga that clearly evolved separately from the chromists. I shall discuss molecular and other evidence concerning the number of separate origins of metaalgae and eualgae and the nature of their protozoan and photosynthetic ancestors.

Changes in Distribution of Dinitrogenase Reductase Subunits of Trichodesmium in Response to Time of Day and Iron Nutrition

Karen M. Elardo. Portland State University, Department of Biology, Portland, OR 97207

Colonies of Trichodesmium thiebautii, a marine cyanobacterium, were analyzed for the presence or absence of a modified (higher molecular weight) subunit of dinitrogenase reductase in two different environments, the Caribbean Sea and the Sargasso Sea. The modified subunit corresponds to the inactive form of the enzyme. Differences in migration of the subunits were observed between the two environments. Diel patterns in subunit distribution were evident in the Caribbean Sea and mostly absent in the Spring Sargasso Sea colonies. The Caribbean colonies were monitored for protein content that complements previous studies on nitrogenase activity. Incubations with FeCl₃ and Saharan dust had different effects on the subunits. Colonies treated with dust had a small amount of active form of the enzyme after "turn off" while FeCl₃ resembled the control. Iron in dust particles appear to be more accessible to colonies. There is a distinct difference in colony morphology between the two sites and a difference in distribution of the subunits of dinitrogenase reductase.

POTENTIAL PHARMACEUTICALS FROM MARINE ALGAE

W. H. Gerwick, M. A. Roberts, J.L. Chen, Z.D. Jiang, C. Mrozek, D. Nagle, P. Proteau, M. Wise, College of Pharmacy, Oregon State University, Corvallis, Oregon 97331.

Our natural products chemistry program examines marine algae, both microphytes and macrophytes, from the wild and from culture collections for potential pharmaceuticals. This work has already surveyed 69 microalgae (Cyanobacteria-28 samples; Cryptophytes-7 samples; Chrysophytes-34 samples) and 617 macroalgae (Chlorophytes-87 samples; Rhodophytes-405 samples; and Phaeophytes-125 samples).

The cell material, as well as the medium from cultured specimens, is processed to provide lipid and aqueous soluble extracts. The crude extracts are tested for anticancer, antiviral, and antimicrobial activity through differential assays performed in house and at SYNTEX laboratories, Palo Alto, CA. Algae whose crude extracts show bioactivity are then scaled-up to large batch culture (50+ L.) or recollected from the wild to obtain sufficient material for active fraction characterization.

The pure and active compounds are characterized and their precise molecular structures determined using a variety of spectroscopic and wet chemical methods. These new substances are then provided to various laboratories for more detailed pharmacological evaluation with the eventual goal being the development of a new drug or research biochemical.

CHARACTERIZATION OF THE MAJOR CAPSID PROTEIN AND CLONING OF ITS GENE FROM ALGAL VIRUS PBCV-1

Michael V. Graves and Russel H. Meints. Department of Botany and Plant Pathology, and Center for Gene Research and Biotechnology, Oregon State University, Corvallis, Oregon 97331, USA

PBCV-1 is one of a group of large dsDNA viruses which infect exsymbiotic *Chlorella*-like green algae. These viruses are easily assayed since they will form plaques on algal lawns. Much heterogeneity exists among these viruses based upon DNA restriction polymorphisms, differences in protein banding patterns, as well as other criteria.

One characteristic which these viruses have in common is the apparent molecular weight, 54,000d, of the most abundant structural protein (Vp54). Vp54 is a glycoprotein, exists as a dimer of MW ~ 104,000 d, and is exposed on the surface of the virus which indicates that it is the major capsid protein. Further study of this protein from the virus PBCV-1 shows that the dimer is not the result of disulfide bonds and that the protein first appears approximately 90 minutes post infection which agrees well with the beginning of virus assembly as seen in electron micrographs. The gene encoding Vp54 has been cloned and sequenced. Initially, a region of the gene was amplified using the polymerase chain reaction (PCR) primed with oligonucleotides derived from the N-terminal amino acid sequences of purified protein and cyanogen bromide cleavage fragments. The PCR product was used as a probe to map the location of the gene to PBCV-1 genomic PstI restriction fragment P8. A 1314-bp open reading frame (ORF) was identified which contained the predicted coding regions from the derived amino acid sequences. The peptide encoded by this ORF had a predicted molecular weight of 48.2 kDa and contained six putative N-linked and 63 putative O-linked glycosylation sites. Primer extension analysis indicated that transcription started 14 bp 5' to the ATG. The gene for Vp54 was transcribed late in infection and this transcript was the most abundant viral RNA present in infected cells.

IMPROVED FEASIBILITY FOR DEVELOPMENTAL GENETICS IN *ACETABULARIA*: LESS CULTURE MAINTENANCE, SHORTER CELL LIFE CYCLE & IMPROVED MATING EFFICIENCY

Brenda Hunt, Andrew Zeller, Adam Wexler, and Dina F. Mandoli, Department of Botany KB-15, University of Washington, Seattle, Washington 98195 USA

Few mutants of *Acetabularia acetabulum* (L.) Silva (Chlorophyta), a classic unicellular model for cell biology since the 1930's, have ever been selected and most of these have been lost due to difficult and labor intensive cell maintenance. We will review the factors which have hindered progress in *Acetabularia* genetics (see Green 1976, In: The Genetics of Algae (RA Lewin, Ed) pp. 236-256 UC Press) and discuss the rationale for our multidisciplinary approach to this long-standing problem. To make genetics feasible in this giant alga, we have 1) improved mating efficiency from $\leq 1\%$ to $\geq 62\%$ (Larsen & Mandoli, in preparation); 2) reduced the juvenile phase from 45 to 4 days by adjusting growth conditions (Wexler & Mandoli, unpublished); 3) shortened the time to cap initiation from 16 to ≤ 6 weeks by optimizing the seawater composition (Hunt & Mandoli, unpublished); and 4) developed methods which facilitate handling and growth of millions of cells at a time (Zeller & Mandoli, submitted). Details of our more recent developments in each of these four areas will be discussed. We will also discuss the developmental mutants we have selected using our new methods.

THE EFFECT OF CURRENT SPEED ON NUTRIENT UPTAKE BY *MACROCYSTIS PYRIFERA*

Catriona L. Hurd, Paul J. Harrison and Louis D. Druehl¹. Department of Oceanography, University of British Columbia, Vancouver B.C. V6T 1Z4 and ¹Bamfield Marine Station, Bamfield, B.C., CANADA.

In conditions of low water motion, the availability of inorganic nutrients to macroalgae may be limited by the formation of a nutrient deplete diffusion boundary layer at the thallus surface. The aim of this work was to establish whether morphological characteristics such as a corrugated thallus or marginal spines serve to produce turbulent flow at the thallus surface and thereby reduce the thickness of the boundary layer. Plants possessing such characteristics may be better able to acquire nutrients in areas of low water motion. This poster reports the preliminary results of a study to determine the effect of current speed on nutrient uptake by *Macrocystis integrifolia*, collected from an exposed site. Whole blades of *Macrocystis integrifolia* were preconditioned in nutrient deplete seawater for 24 h and then placed in a low volume (46 L) laminar-flow tank, at 12 °C, with an irradiance of 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The rate of uptake of nitrate and phosphate from seawater containing initial concentrations of 25 μM and 2.0 μM respectively, was measured at flow rates ranging from 0 - 10 cm s^{-1} . For the range of flow rates tested, uptake rates increased at a constant rate with increasing flow rate.

Water Relations and Acetylene Reduction Rates in Cyanobacterial Crusts in the High Arctic R. Lennihan and E. Van Volkenburgh

Nitrogen limits plant growth in many northern terrestrial ecosystems. Cyanobacteria may provide substantial amounts of fixed nitrogen to these landscapes, and it is therefore of interest to understand the factors limiting nitrogen fixation. The single most limiting factor is water. Most field studies of nitrogen fixation express water on a "water content" basis, leaving the nature of possible water potential limitations and the relationship of water content to water potential unclear. This study examines the interrelationship of these parameters in cryptogamic crusts or patenas in the Canadian High Arctic, in an effort to determine whether water content or water potential is a better predictor of nitrogen fixation. We recorded water content, water potential and acetylene reduction rates of "soil" surfaces or cryptogamic crusts in wet sedge meadows on Truelove lowland, Devon Island, N.W.T. (4-10 meters a.s.l., 75 39 N, 84 24 W) through the greater part of the growing season.

Peak acetylene reduction rates ranged from 40 to 80 μmol of acetylene reduced $\text{m}^{-2} \text{hr}^{-1}$ in three meadow sites. Acetylene reduction by cryptogamic crusts is not affected by moisture contents above 300% moisture by dry weight: corresponding to water potentials near zero. Dramatic reductions in acetylene reduction rates occurred in one site when water content fell below this level, with accompanying decreases in water potential ranging from -1 to -4 bars. This event occurred in late July and was followed by rainfall, which restored the acetylene reduction rates to higher levels.

The results of our study confirm that nitrogen fixation is sensitive to decreases in the availability of water. Very slight decreases in matric water potential and water contents below 290% occur concurrently with significant decreases in nitrogen fixation. The cyanobacteria and other nitrogen fixing prokaryotes occur at the interface of the soil and the atmosphere, and are subject to enormous gradients in water potential and water content. Our data indicate that our crust sections are not thin enough to account for this gradient. Within the limits of our resolution, we feel that water content is a better predictor of nitrogen fixation than water potential.

The Reproductive Life Histories and Morphology of *Audouinella purpurea* (Acrochaetiaceae, Rhodophyta) and *Pilayella littoralis* (Ectocarpaceae, Phaeophyta) under low light conditions.

Eric M. Justesen. The Department of Biological Sciences, Humboldt State University. Arcata, California 95521 U.S.A.

Audouinella purpurea and *Pilayella littoralis* exhibit great morphological variation. The life histories of these algae have never been completed in the field, only in unialgal cultures. I observed specimens *in situ* and attempted to grow them on artificial substrates. These two species were studied in an intertidal cave over a 12 month period. The site was chosen because it supported both species of algae and allowed sufficient access during low tide. Both species have extremely varied morphological characteristics depending on their location in the cave. *A. purpurea* produces tetrasporangia and monosporangia in the winter months (i.e., short photoperiods) and *P. littoralis* produces gametes that fuse in the spring (i.e., longer photoperiods). Fragmentation appeared to be important in the maintenance of algal coverage in the cave. Wave shock, desiccation, and light conditions are believed to be the main factors involved in the morphological variations.

CHEMICAL COMPOSITION OF *PORPHYRA PSEUDOLANCEOLATA*. IN BRITISH COLUMBIA CANADA

Handojo T. Kusumo*, Sandra C. Lindstrom** and Lalit. M. Srivastava*.

* Department of Biosciences Simon Fraser University, Burnaby, B.C. V5A 1S6 Canada.

** Department of Botany University of British Columbia, Vancouver, B.C. V6T 2B1 Canada.

As far as food is concerned, *Porphyra* is economically one of the most important seaweeds in the world. Its high food value is contributed by its protein content and vitamins, minerals, and flavour.

This study attempts to determine some of the major chemical constituents in the native Northwest species, *P. pseudolanceolata* Krishnamurthy, and their seasonal changes. *P. pseudolanceolata* was collected monthly from January to May 1991 at Whiffin Spit, Sooke, British Columbia. Among the carbohydrates, the floridoside, isofloridoside and porphyran contents were 1.78%(dw) 2.19%(dw), and 15.25%(dw), respectively. Their contents increased with time, although floridoside and porphyran showed a slight decrease in May. The content of total amino acids was 25.25% (dw) and that of free amino acids was 0.87% (dw). Total amino acids were dominated by alanine and methionine, whereas the free amino acids were dominated by alanine and threonine. The ash content was 16.1% (dw) with slightly variations from month to month. Percent dry weight was 19.3% with no significant variations observed from January to May. These data have value in determining the potential of Northwest species that could be used as food purposes, and the best time to collect them.

IMPROVED MATING EFFICIENCY IN *ACETABULARIA ACETABULUM* (L.) SILVA (CHLOROPHYTA): RECOVERY OF 60-100% OF THE EXPECTED NUMBERS OF ZYGOTES

Tamara Larsen and Dina F. Mandoli, Department of Botany KB-15, University of Washington, Seattle, Washington 98195 USA

We have improved mating efficiency 60-1,000 fold by optimizing the physiology of gamete release and mating in *Acetabularia*. *Acetabularia acetabulum* (L.) Silva (Chlorophyta), a classic research organism in cell biology, is amenable to genetic studies: each cell can be selfed and outcrossed, produces $\geq 10^6$ progeny per generation and can be transformed via microinjection. However, genetic studies of this cell are rare. Green (1976, In: The Genetics of Algae (RA Lewin, Ed) pp. 236-256 UC Press), suggested that mating efficiency was low because 1) gametangia were dormant for long periods of time (17 weeks: Koop 1977 In: Progress in Acetabularia Research (CLF Woodcock, Ed) pp. 7-18. Academic Press, NY) and 2) subsequent gamete release was asynchronous. We do not see dormancy of gametangia using our current culture methods (Hunt & Mandoli, 1992 J. of Phycology, 28(3), in press). Koop's methods to induce gamete release and mating, result in only 0.1-1.0% mating efficiency in "mass mating" of ≥ 5 cysts (Zeller & Mandoli, submitted). Although, we have found that gamete release can be synchronized by a cold incubation, this does not improve mating efficiency as Green (1976) suggested. We can recover 60-100% of the expected numbers of zygotes from a mass mating by inducing gamete release on specially purified agars. We also have determined that the number of gametes per cyst varies from 500-6,000 in a laboratory strain. We are documenting the half-life of gametes and the mating efficiency in self and outcrosses of cyst pairs from both inbred and wild type strains. Our advances make mutant selection and analysis in *Acetabularia* feasible.

Diminished water quality downstream of remotely located, but recreationally accelerated, wilderness development: end-of-pipe vs watershed-management.

Lucey, Wm.P., Bruce Jeffs, Kenneth Congdon, Alan P. Austin, Brent Moore.

A four year field study (1988-1991), using semi-natural stream-troughs, modelled and quantified natural and sewage-generated nutrients (from the recreational community of Whistler) to the Cheakamus River, British Columbia. The ortho-phosphorus concentration inducing undesirable algal production was used to determine the maximum permissible sewage treatment plant discharge, based upon optimal removal of ortho-P. Revelation of the river's limited capacity to assimilate nutrients resulted in a Liquid Waste Management Plan (LWMP) (replacing the existing discharge permit) which proposed the discharge of sewage effluent, via a 15 km pipeline, directly into the adjacent Squamish River. A stream-trough study, on the latter river, was conducted in the summer of 1991 to assess how the diverted effluent could affect water quality, development of undesirable algal growth, and fish spawning and rearing habitat. The results to date have been used to compare and contrast the resource planning practices of 'end-of-pipe permitting' vs 'the watershed as a management unit'. The LWMP, coupled with recent changes in reservoir operation and proposed development of new hydroelectric facilities, could further reduce water quality and habitat in the Squamish River. As a response to increasing environmental deterioration in these, and other connected catchment basins, a Science Advisory Committee has recommended adoption of a watershed management plan.

BOTTOM-UP AND TOP-DOWN COMMUNITY REGULATION: ARE ROCKY INTERTIDAL FOOD WEBS AN EXCEPTION?

Bruce A. Menge. *Department of Zoology, Oregon State University, Corvallis, OR 97331-2914 USA*

Two general hypotheses proposed to explain community regulation are the "top-down", or food-chain dynamics model and the "bottom-up", or nutrient/productivity model. Top-down models argue that communities are regulated by trophic interactions or "trophic cascades", and thus that patterns of community structure depend primarily on the interplay between predation and competition. This model has been applied to aquatic communities, especially those on marine rocky shores. Bottom-up models argue that communities are regulated by the level of nutrients/productivity, and thus that community structure depends on how many trophic levels increasing nutrients/productivity can support. This model has been applied to freshwater and terrestrial communities. Here, I suggest that these models are complementary rather than alternatives. Studies of predation intensity on rocky shores at Boiler Bay and Strawberry Hill, Oregon suggest that variation in the strength of these interactions depend in part on variation in phytoplankton productivity. Thus, the importance of top-down regulation may vary with variation in bottom-up factors. Complete understanding of variation among communities will depend in integrating knowledge of both bottom-up and top-down processes into a unified model of community regulation.

COMPARISON OF THE GENE STRUCTURE AND SEQUENCE FROM VIRUSES INFECTING CHLORELLA-LIKE GREEN ALGAE

Vanda A. Moniaga, Michael V. Graves, and Russel H. Meints. *Department of Botany and Plant Pathology, Center for Gene Research and Biotechnology. Oregon State University, Corvallis, Oregon 97331-2906, USA.*

We have isolated large dsDNA viruses from unicellular, eukaryotic Chlorella-like green algae. These viruses are ubiquitous in nature and specifically infect exsymbiotic Chlorella. A highly expressed gene was cloned and sequenced from viruses PBCV-1 and OR-1. This gene produces the most abundant peptide in an *in vitro* translation system of apparent molecular weight of 33 kDa, and was chosen for comparative study due to its degree of conservation within the family (Phycodnaviridae) of exsymbiotic Chlorella viruses. Between PBCV-1 and OR-1 the two genes differed by 14 bp leading to two conservative amino acid substitutions. Significant differences in sequence were noted in the long leader sequence present 5' to the genes. This region contained 10 AAAC repeats in OR-1 and 16 of the same repeats in PBCV-1. The region near the transcriptional start site has the potential to form a hairpin loop. Both viruses contained several similar repeats at the same location in the 3' region. The most striking difference in this genomic region between these viruses was the absence of an additional open reading frame (ORF 1) from OR-1.

STRUCTURE AND EXPRESSION OF THE psbD/C
OPERON IN A CHROMOPHYTIC ALGA.

Tracie Nadeau, Klaus Valentin, and Rose Ann Cattolico.
Department of Botany, University of Washington, Seattle, WA
98195.

The psbD and psbC genes encode for the D2 protein and 44kd chlorophyll-binding protein of photosystem II, respectively. These genes are highly conserved among cyanobacteria and chlorophytic plants, and therefore may be useful phylogenetic markers. Organizational features which vary between taxa include an overlapping region of the 3' end of the psbD gene with the 5' end of the psbC gene, as well as insertions and deletions in the genes. Variations in these features can be regarded as signatures of different taxa. For example, there is a one amino acid insertion at the carboxy terminus of the D2 protein in terrestrial plants that is absent in cyanobacteria and a unicellular chlorophytic alga. The monophyletic versus polyphyletic origin of chloroplasts is still under debate. Very little data exists at the molecular level for non-chlorophytic plastids, thus we decided to explore the organization of the psbD/C operon in the chromophytic alga Olisthodiscus luteus. Furthermore, chloroplast genes are predominantly post-transcriptionally regulated in chlorophytic plants, whereas in O. luteus some photosynthetic genes (rbcL/S, psbA) are light regulated at the transcriptional level. We are currently studying the regulation of the psbD/C operon in this alga.

NOVEL OXYLIPINS FROM *CONSTANTINEA SIMPLEX* SETCHELL
(WEEKSIACEAE, RHODOPHYTA).

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Eicosanoids, including prostaglandins, hepoxilins, and leukotrienes, have been shown to be mediators of vital physiological processes in mammalian systems. A number of disease states including cardiovascular disease, inflammatory disorders, and gastric ulceration, have all been linked to eicosanoid mediated events. The importance of arachidonic acid metabolism in the marine environment has become the subject of considerable interest in recent years.

Several experiments designed to investigate the possible involvement of a proposed allylic epoxide intermediate in the biosynthesis of *Constantinea's* eicosanoids have been performed. Our work contains proof of the first reported cell-free production of novel cyclopropyl lactone fatty-acid metabolites from marine algae. Labeling studies utilizing acetone powder enzyme preparations incubated in the presence of $^{18}\text{O}_2$ contribute additional support to our proposed mechanism for the biosynthesis of these novel eicosanoids. We have also isolated a series of new cyclopropyl containing vicinal-diol constanolactone analogues from *Constantinea simplex*. Stereochemical and conformational information obtained from newly formed derivatives of the constanolactones, only recently isolated as their free alcohol natural products, provide additional evidence for the intermediacy of an allylic epoxide in the formation of the constanolactones.

ALGAL EPIPHYTE, GRAZER AND EELGRASS INTERACTIONS IN A SUBTIDAL EELGRASS MEADOW: A PROGRESS REPORT.

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This paper reports progress on development of, estimation of parameters for, and testing of a mathematical model of observed seasonality of grazers on algal epiphytes in a subtidal eelgrass (*Zostera marina*) meadow. Microcosm experiments suggested that the herbivorous gastropod *Lacuna variegata* is capable of preventing epiphytic overgrowth of the host eelgrass leaves. Eelgrass, epiphyte, and grazer biomass, epiphyte productivity, and major abiotic factors are measured monthly in an eelgrass meadow near Brown Island, Friday Harbor, Washington. Eelgrass biomass peaks at up to 404 g.d.w./m² from midsummer to early fall, and is at a minimum as low as 72 g.d.w./m² in January and February. Epiphyte biomass peaks in the late summer and early autumn as high as 202.3 g.d.w./m². An expected spring bloom was consumed by *Lacuna*, which, in turn, has a biomass peak in April and remains abundant through the summer before declining during the fall. Epiphyte productivity peaks in the spring and autumn ($P_{\max} = 10.27$ mgC/g.d.w./h), declining sharply in midsummer and midwinter ($P_{\max} = 3.46$ mgC/g.d.w./h). Total nitrogen and nitrate measured in the water column (ranging from 7.39-28.41 and 8.06-28.83 μ M, respectively) appears to be negatively correlated with epiphyte biomass. Ammonium concentration (0.05-1.69 μ M) is positively correlated with epiphyte productivity. These data will be used to calibrate and test a system of two ordinary differential equations describing the epiphyte grazer interaction. This system has been shown to be stable when parameters are constrained to biologically meaningful values.

IMPORTANCE OF CURRENT VELOCITY AND NUTRIENT FLUX IN STRUCTURING PERIPHYTON COMMUNITIES.

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Structure and function of lotic periphyton communities is influenced by many biotic and abiotic parameters. Current and nutrient concentration are known to be important in structuring periphyton communities, but the mechanisms by which this occurs are not fully understood. Water velocity affects periphyton communities physically (shearing forces) and chemically (nutrient fluxes), but the relative importance of these factors has not previously been assessed. An experiment was conducted in outdoor, stream-troughs in an attempt to partition these factors. Velocities of 6, 9, and 12 cm/sec were used to assess the effects of current on periphyton structure (biomass and species composition) and function (nutritional content). In two additional treatments, designated 6N+ and 9N+, nutrients (nitrate and ortho-phosphate) were added to stream-troughs with velocities of 6 and 9 cm/sec to approximate the nutrient flux (μ gN&P/time) occurring in the 12 cm/sec velocity treatment. Periphyton standing crop increased with increasing current velocity (chlorophyll *a* maxima of 0.029, 0.057 and 0.071 mg chl *a*/dm for treatments 6, 9, and 12 cm/sec respectively, after 35 days). The treatments 6N+, 9N+ had a greater biomass accrual than the 12cm/sec treatment. Treatment 9N+ had the highest periphyton standing crop of all treatments (0.117 mg chl *a*/dm after 35 days), whilst the biomass maximum for treatment 6N+ was 0.094 mg chl *a*/dm and occurred after 21 days, 2 weeks prior to the biomass maximum in any other treatment.

ECOPHYSIOLOGY AND ULTRASTRUCTURE OF HAIRS ON THE AGAROPHYTE RED ALGAE GELIDIUM VAGUM AND GRACILARIA PACIFICA.

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Hairs on the agarophytes Gelidium vagum and Gracilaria pacifica can be very conspicuous reaching densities of 147 hairs mm^{-2} and 162 hairs mm^{-2} , respectively. On Gelidium hairs can increase the absorptive surface by 45%, while hairs on Gracilaria increase the surface area of the alga by 180%. Utilizing dilute concentrations of the vital dye neutral red we have shown directly that the hairs are involved in uptake and transport of solutes to subtending cells. Preincubation of thalli in KCN blocks uptake demonstrating that the process is active. Hair induction differs between the two species. Hairs can be induced on Gracilaria simply by reducing the nitrogen concentration of the culture medium, whereas hairs will form on Gelidium only after the alga has been subjected to short-day (8L:16D) for an extended period of time. Hairs on both species contain membrane systems that appear to enable them to perform a secretory function. In Gracilaria, secretion, by the hairs or other cells, may play an important role in the intertidal sand habitat. When hairs are present on the alga a layer of polysaccharide almost as deep as the hairs surrounds the alga. This polysaccharide layer greatly reduces exchange between the alga and the surrounding medium.

ALGAL-BASED FOOD WEBS IN RIVERS.

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In rivers that are sunlit, rock-bedded, and clear at low flow, the dynamics of attached algae strongly influence ecological communities. Fish, in turn, have strong effects on algal dynamics that may be mediated directly, or through two or three trophic levels in river food webs. In south-central Oklahoma creeks, in Ozark rivers, and in rain forest streams of central Panama, algae-grazing fishes dominate herbivore guilds. River substrates are barren where these fish graze attached algae, but lush standing crops of algae develop where they are excluded by piscivorous fish or birds. Experiments reveal rapid responses (within weeks) by algae and algivorous fish to release from their respective consumers. In rivers lacking specialized algivorous fish, invertebrates commonly dominate grazer guilds. In a northern California river, carnivorous and omnivorous fish suppress algae indirectly by consuming predators of algivorous chironomid larvae. In experimental enclosures with fish, algal turfs were reduced and the formation of floating algal mats was virtually eliminated, in contrast to turf persistence and mat production in fish enclosures. Algal production, particularly the formation of floating mats, appears to greatly enhance the emergence of aquatic insects from the river, and may have substantial impact on the amount of insect production routed from the river to consumers in the surrounding old growth conifer forest.

TRANSCRIPTIONAL REGULATION OF PHOTOSYNTHETIC GENES DURING A DIEL CYCLE IN THE CHROMOPHYTIC ALGA *O. LUTEUS*

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Chromophytic algae are major contributors to primary production in marine ecosystems, yet we know little about the molecular details of how the process of photosynthesis is regulated in response to varying environmental conditions. The chromophyte *Olisthodiscus luteus* represents a model system for such analysis since it can be cultured axenically and grows synchronously on a 12L:12D light regime. We have assayed transcription of chloroplast encoded Calvin cycle genes *rbcl*, *rbcS* (cotranscribed as a 2.6 kb RNA), and the photosystem gene, *psbA* (1.3 kb transcript) during a diel cycle. Northern analysis of RNA indicated that a dramatic fluctuation in steady-state levels of both mRNAs occurs with a minimum in the dark approximately 20 fold lower for both transcripts than in the light. To determine whether the fluctuation in RNA level was due to altered transcription initiation or stability of mRNA, a plastid run-on assay was established. In this assay the amount of transcription initiation was monitored by the elongation of nascent transcripts from isolated lysed plastids in the presence of ^{32}P -UTP. The same fluctuation was seen in run-on assays for *psbA* and *rbcl*, indicating that the critical step for regulation of mRNA levels of these genes is transcription initiation. The signal which directs this fluctuation may be a direct light cue or an endogenous factor, such as a circadian rhythm or cell cycle control. These hypotheses are being tested.

THE IMPORTANCE OF IRON TO OPEN OCEAN PHYTOPLANKTON

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The amount of iron required by marine phytoplankton varies widely and depends on their physiological state. Estimates for the ratio of nitrogen to iron in phytoplankton range from 250 to 10,000 mol N:mol Fe. An inventory of iron containing compounds explains some of this variability, especially the very high iron requirement for the marine nitrogen-fixing cyanobacterium *Trichodesmium*. Processes that require a high investment of cellular iron, such as nitrogen fixation, nitrate reduction to ammonium or light harvesting, can be rate limiting for growth under low iron conditions. Regulation of the relative allocation of iron to these metabolic processes is necessary to optimize cell growth rates. Because of the links between iron nutrition and physiological state, there are significant interactions in the geochemical cycles for nitrogen, carbon and iron.

IRON ACQUISITION BY SIDEROPHORES:
A COMPARISON OF STRATEGIES AVAILABLE TO
FRESHWATER AND MARINE CYANOBACTERIA

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Many freshwater cyanobacteria respond to iron limitation by producing extracellular siderophores. This strategy seems to be absent from open ocean cyanobacteria. The mechanism of response to low iron is well described in *Escherichia coli*. In an attempt to determine if a similar set of mechanisms were operating in the cyanobacteria, we probed chromosomal DNA from several strains of freshwater and marine cyanobacteria with a fragment of the ferric uptake regulation gene (*fur*) from *E. coli*. There was some evidence for hybridization in the species that produced siderophores. Cultures of marine *Synechococcus* did not make siderophores even under severe iron limitation nor did their DNA hybridize to the *E. coli fur* gene probe. A *fur*-like system in some cyanobacteria may provide a plausible explanation for the sensitivity of these algae to Mn toxicity.

Ecological differences between isomorphic generations of *Iridaea splendens* (Gigartinales, Rhodophyta)

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Investigations into ecological differences between generations have approached this question by sampling populations over time to determine generation abundance. More recently, investigators have been using an experimental approach. De Wreede and Green (1990) found that for intertidal populations of *Iridaea splendens* in Vancouver there was, over 3 years of sampling, a high relative abundance of gametophytes in the spring/summer and sporophytes in the fall/winter. The research presented here is part of a larger population data set in which gradients in time and space (inter - vs subtidal; more vs less wave exposed) have been sampled for the abundance, biomass and reproductive condition of each generation of *I. splendens* and other taxa in the Gigartinales. These new data will be compared with existing data and be used to make new or modify existing hypotheses. An experiment that tests one of these hypotheses will also be presented.

MICROBIAL FOOD WEBS: HERBIVORY ON THE MICROSCALE

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During the past 10-15 years, there has been a revolution in concepts of the structure and functioning of pelagic food webs, in which increased attention has been focused on $< 5 \mu\text{m}$ sized ('ultra') phytoplankton and on their chief predators, phagotrophic protists. It now appears that herbivorous flagellates and ciliates, mostly cells from 5 to 50 μm in size, are a vital trophic link between 'ultra' phytoplankton and metazoan grazers. Since $< 5 \mu\text{m}$ phototrophs represent a large fraction of total phytoplankton standing stock biomass and primary production in oligotrophic ocean and lake water, the role of phagotrophic protists as herbivores should be greater than that of microcrustaceans in such systems. Examples of protistan herbivores found in Atlantic and Pacific coastal waters, visualized via epifluorescence microscopy, will be shown.

AMMONIUM ADDITIONS CAUSE AN INCREASE IN THE DARK CARBON FIXATION RATE FOR IRON-LIMITED CYANOBACTERIA GROWING ON NITRATE

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Cultures of the marine cyanobacteria *Synechococcus* WH6501 and WH7803 were grown in AQUIL. These cultures were assayed for carbon fixation rate while still in active growth phase, before the depletion of nitrate from the media. Ammonium, ($5\mu\text{M NH}_4\text{Cl}$), was added prior to three-hour ^{14}C uptake incubations in both light ($70 \mu\text{mol photons m}^{-2}\text{s}^{-1}$) and dark. Ammonium addition increased the rate of dark carbon fixation in iron-limited (10^{-8} M Fe) cells from 22-50% but had no effect on iron-replete cells (10^{-7} M Fe). Similar experiments were performed with the freshwater *Synechococcus* strain PCC7942. This strain had much higher dark carbon fixation rates with added ammonium. We hypothesize that ammonium addition relieves the limitation of nitrate and nitrite reduction to ammonium leading to faster synthesis of amino acids.

THE EVOLUTION OF ALGAL PLASTIDS AND THEIR GENOMES

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The endosymbiotic origin of the plastids is generally accepted. Recent results indicate that at least two such endosymbiotic events may have led to plastids of Rhodophyta and Chlorophyta. Chromophytic plastids appear to have evolved by secondary endosymbioses of unicellular red algae in colorless, eucaryotic host cells. These hypotheses imply that rhodophytic/chromophytic and chlorophytic plastid genomes may differ not only at the level of gene sequence homologies but also at the levels of gene content and gene regulation. Indeed, sequence and gene expression analysis of rhodophytic and chromophytic plastid genomes reveals significant differences compared to the situation in chlorophytic plastids. On the basis of these results a model is suggested for the evolution of the plastid genome.

AN INEXPENSIVE TANGENTIAL FLOW SYSTEM FOR CONCENTRATION OF NANO- AND PICOPLANKTON IN OLIGOTROPHIC WATERS

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Molecular methods for examining microbial diversity in open waters often require concentration of large volumes of seawater either to obtain enough material for DNA or RNA extraction or to insure adequate sampling of the community. Here we examine a method of tangential flow filtration which is less expensive than previously described systems (Barthel et al. 1989, J. Plankton Res., Giovannoni et al., 1989, Appl. Env., Micro.) and which has the advantage of a small "dead volume" in the cartridge. It is useful for molecular studies, photosynthesis measurements, and for production of large volumes of filter sterilized water for experimental use.

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