6-1-2005

Collaborative Reseach: Nitrogen Limitation and Ultraviolet Stress in Marine Macroalgae

J. Malcolm Shick
Principal Investigator; University of Maine, Orono, shick@maine.edu

Carl Grobe
Co-Principal Investigator; Westfield State College

Final Report for Period: 09/1999 - 08/2003 **Submitted on:** 06/01/2005
Principal Investigator: Shick, J. Malcolm . **Award ID:** 9907305
Organization: University of Maine

Title:
Collaborative Reseach: Nitrogen Limitation and Ultraviolet Stress in Marine Macroalgae

Project Participants

Senior Personnel

Name: Shick, J. Malcolm
Worked for more than 160 Hours: Yes
Contribution to Project:

Name: Shick, J. Malcolm
Worked for more than 160 Hours: Yes
Contribution to Project:
Became PI when Davison left University of Maine, and the bulk of the remaining funds for research were subcontracted to him.

Post-doc

Name: Fegley, Jill
Worked for more than 160 Hours: Yes
Contribution to Project:
Dr. Jill Fegley was hired as a post-doc on April 1, 2001 for a one-year period. This position is partially funded from the grant and partially funded by the University of Maine.

Graduate Student

Undergraduate Student

Name: Kershaw, Brian
Worked for more than 160 Hours: Yes
Contribution to Project:
Brian is an undergraduate from Westfield State College who is spending the summer at the University of Maine's marine station (Darling Center) working on the project.

Name: Bristol, Eric
Worked for more than 160 Hours: Yes
Contribution to Project:
Eric Bristol is an undergraduate biology major from Westfiedl state college who is participating in the 2001 summer research at the darling Marine Center. His roles include field support and helping maintain cultures and general laboratory assistance.

Name: Stavros, Heather
Worked for more than 160 Hours: Yes
Contribution to Project:
Field and laboratory support of research

Name: Wood, Emmanda
Worked for more than 160 Hours: Yes
Contribution to Project:
Field and laboratory support of research

Name: Ferris, Dianne

Worked for more than 160 Hours: Yes

Contribution to Project:
Laboratory support of research

Name: Caligara, Chiara

Worked for more than 160 Hours: Yes

Contribution to Project:
Field and laboratory support of research

Technician, Programmer

Other Participant

Name: Grobe, Carl

Worked for more than 160 Hours: Yes

Contribution to Project:
Dr. Grobe is a co-PI on the project. The project is supported by two grants, one to Westfield State College (Grobe) and the other to the University of Maine (Davison and Shick). However, these grants support a single integrated research project, and all of the activity supported through both Westfield State College and the University of Maine is described in this report.

Name: Davison, Ian

Worked for more than 160 Hours: Yes

Contribution to Project:
Davison was the original lead PI on this grant. He left the Univeristy of Maine in 2001.

Research Experience for Undergraduates

Organizational Partners

Westfield State College

The research project is a collaborative effort between Westfield State College (Carl Grobe) and the University of Maine (Ian Davison, J Malcolm Shick). There is a single research project supported by grants to both institutions. The research was conducted at the University of Maine's campus in Orono and at the Darling Marine Center (Walpole, Maine).

Academy of Natural Sciences, Benedict Estuarine Research Center

This is the institution to which Ian R. Davison, the original lead PI on this grant, moved during the course of the grant. Much of the analytical work was subcontracted to him at that institution.

Centre Scientifique de Monaco

Shick collaborated with scientists at CSM on experiments involving effects of UV and inorganic nitrogen supplementation on the accumulation of natural sunscreens (MAAs, the same compounds as present in red macroalgae) in corals. CSM provided aquarium and laboratory facilities, housing, and research supplies.

Other Collaborators or Contacts

At the Centre Scientifique de Monaco: Prof. Denis Allemand; Dr. Christine Ferrier-PagÞs; Dr. Renaud Grover.

Activities and Findings

Research and Education Activities:

This project examined the interaction between summer nitrogen limitation and ultraviolet radiation (UVR) on growth and metabolism of brown and red seaweeds, and additionally the effects of these environmental factors on the concentrations of natural sunscreens (mycosporine-like amino acids, MAAs) in the red alga, Chondrus crispus.

Carl Grobe and five undergraduate students from Westfield State College, and one student from the University of Nice, spent two months (mid-June to mid-August) in each of three field seasons at the University of Maine's Darling Marine Center. Ian Davison (original lead PI) and Jill Fegley (post-doc) also participated in the field work, to varying extents.

The final growth experiments were completed in August 2002 and the biochemical analysis of the large number of samples was not completed until July of 2004. Davison changed positions three times in the course of the grant, and a post-doc whom he hired and trained in April 2001 left to take a faculty position in September of that year. This disrupted his plan to maintain a laboratory at the University of Maine to complete this project, and he was unable to hire a technician in his new laboratory in Maryland until April of 2002.

The planned experiments on cultured red algae under controlled laboratory conditions in a growth chamber and using a solar simulator could not be carried out because Davison left the University of Maine, and the postdoc (Fegley) left to accept a permanent academic position soon thereafter. Because Shick did not have experience in algal culture, he participated in parallel studies on the effects of acute exposure to UVR and nitrogen enrichment on laboratory cultured corals and analyzed the MAAs (natural sunscreens) in these specimens.

Findings:

Algae were grown for 4-6 weeks in July and August of 2000, 2001 and 2002 in ambient seawater (? 50 ÁM nitrate) and exposed to 50% ambient sunlight (?UVR). All species of brown algae were nitrogen limited, exhibiting increased growth when additional nitrogen was supplied, and the response to UVR reflected natural zonation patterns. Growth of the intertidal Fucus vesiculosis was unaffected by UVR, whereas the deep water Agarum cribosum was severely inhibited and unable to grow at this UVR dose. Growth of Laminaria saccharina, which occurs at intermediate depth, was reduced under UVR, with nitrogen-limited algae being more susceptible than those grown under nitrogen-replete conditions. These data indicate that the biological response to UVR depends on factors such as nutrient availability. Physiological responses such as photosynthesis and Fv/Fm were poor predictors of the growth response.

Samples taken in the field for measurement of production of reactive oxygen species (ROS) indicated that production in Laminaria increased gradually with decreasing depth (increasing light), whereas Agarum exhibited a massive increase above 4 m (30% surface light), the upper distribution limit of this species. UVR increased the production of ROS in all species (compared to natural sunlight minus UVR), with the greatest increase in A. cribosum. The levels of antioxidants (e.g., ascorbate) and protective enzymes (e.g., superoxide dismutase) reflected the UVR susceptibility of the three brown algae (i.e., contents/activities were ranked Fucus>Laminaria>Agarum), suggesting that UVR-induced photo-oxidation is important in the response to UVR. However antioxidants and protective enzymes did not acclimate in response to UVR, but did increased in the N-enrichment treatments, suggesting that nitrogen enhances the ability of these algae to withstand UVR-induced oxidative stress.

All of the red algal genera examined (Chondrus crispus, Porphyra umbilicalis, Phycodrys rubens, and Palmaria palmata) proved to be highly sensitive to both UVR or nitrogen limitation. For most genera, growth was greater in the nitrogen-supplemented treatments. In contrast to the other three species, final Fv/Fm in C. crispus was significantly affected by nitrogen but not by UVR. An initial decline in fluorescence in response to high light was similar in all treatments; however, recovery was faster in under nitrogen-supplementation. Gross Pmax significantly increased with enriched nitrogen in Chondrus crispus, but was not affected by UVR. Respiration was not significantly affected by UVR or nitrogen in any species, although our data showed a pattern of decrease with UVR. Treatment-specific differences in the levels of antioxidants and the activities of antioxidant enzymes varied for each enzyme and antioxidant. Ascorbate, ascorbate peroxidase, and glutathione reductase did not respond to nitrogen or UVR, and there was no interaction between these factors. Superoxide dismutase and catalase increased with increased nitrogen. Glutathione increased with nitrogen and decreased with UVR. Percent tissue nitrogen mirrored nitrogen status, with a significant response to nitrogen enrichment but not to UVR. There was an interaction effect in nitrate reductase, with a significant reduction in activity under the ambient +UVR treatment when compared with the ambient minus UVR treatment.

In Chondrus, concentrations of MAAs were unaffected by experimental exposure to UVR, probably because the test specimens had been previously exposed to UVR in the field, as evinced by their high and variable concentrations of MAAs, which were similar to those reported in European populations of this species freshly collected from nature. This may account for the relative insensitivity of this species to UVR seen in other measures such as photosynthesis and respiration. N-enrichment did, however, increase the concentration of shinorine and the total MAA concentration significantly, again indicative of N-limitation under ambient conditions. Because shinorine accounted for a maximum of only 20% of the total MAA concentration in these specimens, the significant effect of N-enrichment on total MAAs also seems owing to not-quite-significant increases in palythine and usujirene (overall the most concentrated MAAs).

Parallel studies on UV-na´ve specimens of the coral Stylophora pistillata cultured in the laboratory (at the Centre Scientifique de Monaco) showed clear effects of both UVR and N-enrichment (10 ÁM ammonium) on MAA concentration. Starved corals not supplemented with ammonium accumulated MAAs and conserved them disproportionally compared with declining protein and chlorophyll levels during progressive starvation, which indicates the priority of maintaining UV defenses. Genotypically distinct colonies had different responses to UVR when not exposed to elevated ammonium: fragments of one colony produced MAAs at the expense of chlorophyll, while the other maintained levels of chlorophyll but accumulated less MAA. Ammonium-enrichment consistently affected only the primary MAAs synthesized by the zooxanthellae (endosymbiotic dinoflagellates) in the corals, not the secondary MAAs produced in the coral hostÆs tissues from primary MAAs released by the algae. Kinetic experiments indicated that exposure to UVR upregulated the enzymes involved in MAA-biosynthesis, rather than UVR being required for the activity of a photoenzyme. ROS produced during exposure to UVR were implicated as being involved in this upregulation. Once synthesized, MAAs appeared to regulate their own biosynthesis, likely by attenuating the UV-signal reaching the zooxanthellae. PAR was required for the photosynthesis-dependent, UV-triggered accumulation of MAAs in the coral. MAAs accumulated more rapidly when the UV-dosage rate (UV irradiance level) increased, a response that may be important during short-term increases in UVR in nature, such as during doldrum conditions.

The planned experiments on red algae under controlled laboratory conditions (similar to those conducted on corals) could not be carried out because Davison left the University of Maine, and the postdoc (Fegley) left to accept a permanent academic position soon thereafter.

Training and Development:
The involvement of five Westfield State College undergraduates provided them with an opportunity that was otherwise unavailable (or at least severely limited) at this teaching-oriented institution. The opportunity to be involved in an intensive summer research project provided them with an educational experience to which they would otherwise never have been exposed. All of GrobeÆs students learned SCUBA in order to participate in the project, and received advanced SCUBA and lifesaving training through the Darling Marine Center.

Outreach Activities:

Journal Publications

Davison, I.R, J. Collén and J.C. Fegley, "Reactive oxygen metabolism in the tropical brown alga Dictyota dichotoma.", J. Phycology (supplement), p. 15, vol. 36, (2000). Published abstract,

Grobe, C.W. and I.R. Davison, "Effect of nitrogen and UV on growth and photosynthesis of Laminaria saccharina (Phaeophyta)", Phycologia (supplement), p. 42, vol. 40, (2001). Published abstract,

Shick, J.M., "The continuity and intensity of ultraviolet radiation affect the kinetics of biosynthesis, accumulation, and conversion of mycosporine-like amino acids (MAAs) in the coral Stylophora pistillata", Limnology and Oceanography, p. 442, vol. 49, (2004). Published,

Shick, J.M., C. Ferrier-Pagès, R. Grover, and D. Allemand, "Effects of starvation, ammonium concentration, and photosynthesis on the UV-dependent
accumulation of mycosporine-like amino acids (MAAs) in the coral Stylophora pistillata", Marine Ecology Progress Series, p. , vol. , ( ). Accepted,

Furla, P., J.M. Shick, et (5) al., "The symbiotic anthozoan: a physiological chimera between alga and animal", Integrative and Comparative Biology, p. , vol. , ( ). Accepted,

Davison, I.R., T.L. JOrdan, J.C. Fegley, and C.W. Grobe, "Species-specific responses of brown algal growth and photosynthesis to simultaneous
UVR and nitrogen limitation", J. Phycol., p. , vol. , ( ). Submitted,

Books or Other One-time Publications

Collén, J. and I.R. Davison, "Diurnal changes in photosynthesis and
reactive oxygen metabolism in
Gracilaria tikvahiae (Rhodophyta)", (2003). Book, Published
Editor(s): A.R.O. Chapman, R.J. Anderson, V.J.
Vreeland, and I.R. Davison
Collection: Proceedings of the 17th International
Seaweed Symposium
Bibliography: Oxford University Press, U.K., pp. 395-401

Web/Internet Site

URL(s):

Description:

Other Specific Products

Contributions

Contributions within Discipline:

Our principal contribution is demonstrating that the nitrogen limitation of seaweeds that occurs during summer extends to their antioxidant and sunscreen defenses at a season when solar irradiance (including UV radiation) is highest. Therefore, because solar UVB radiation is predicted to increase for another half-century owing to ongoing depletion of statospheric ozone, the productivity of seaweeds may be affected to a greater extent than predicted simply from the effects of nitrogen limitation on growth. This is also inferred from the depth (UV) dependent sensitivity of growth in some species. The results also implicate reactive oxygen species (ROS) produced endogenously under high irradiance as a contributor to the deleterious effects of bright PAR and high levels of UVR, which enhance ROS production. These effects contribute to the bathymetric zonation of seaweeds.

Complementary studies on vegetatively propagated corals (symbioses between dinoflagellate algae and cnidarian hosts) also reveal nitrogen limitation of sunscreen defenses, specifically, mycosporine-like amino acids (MAAs). By using genotypically distinct colonies, we showed a genetic component to the response of corals to UVR. Although large genotypic variation among endosymbiotic dinoflagellates (zooxanthellae) in corals has been documented by others, less is known of host variability, and mechanistic studies of the physiological manifestations of such variation are just beginning. Apparently UV-exposed zooxanthellae within the host can maintain chlorophyll concentrations or synthesize MAAs, but they cannot maximize both under ambient concentrations of inorganic nitrogen. There seems to be a genotypic contribution to this difference that bears further study. Nitrogen availability represents another resource axis that may contribute to the sensitivity of corals to UV-induced bleaching. Also, UV-induced ROS are implicated in the upregulation of MAA-biosynthesis. Once synthesized, MAAs appear to regulate their own biosynthesis, likely by attenuating the UVR stimulus.

Contributions to Other Disciplines:

Contributions to Human Resource Development:

One of the undergraduate students from Westfield State College who participated in the research has gone on to graduate study, and another is planning to apply. An Italian student who became interested in the research while attending ShickÆs lectures at the University of Nice asked to join the students working in Maine, and participated in the field and laboratory work in 2002; she is currently in a Ph.D. program in Italy. The project also provided six months of support in 2001 for a Post-Doctoral Research Fellow (Dr. Jill Fegley) who later left the project to take a full-time faculty position at the Maine Maritime Academy. The grant supported (on a subcontract) Terry Jordan as DavisonÆs technician after he moved to Maryland, and was instrumental in her decision to attend graduate school; she is currently enrolled in the Ph.D. program at the University of MarylandÆs Horn Point Laboratory.

Contributions to Resources for Research and Education:

Contributions Beyond Science and Engineering:

Categories for which nothing is reported:

Activities and Findings: Any Outreach Activities

Any Product

Contributions: To Any Other Disciplines

Contributions: To Any Resources for Research and Education

Contributions: To Any Beyond Science and Engineering