Final Report for Grant #NAG5-8170
UV-Visible Spectroscopic Methods and Models for Assessment and Monitoring of Harmful Algal Blooms

Principle Investigator:

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Abstract

The development of an enhanced predictive and early warning capability for the occurrence and impact of harmful algal blooms (HABs) would be of great benefit to coastal communities. A critical issue for early detection and monitoring of HABs is the need to detect harmful algal species within a mixed-species phytoplankton assemblage. Possession of UV-absorbing compounds called mycosporine-like amino acids (MAAs) may be one factor that allows HAB species to out-compete their phytoplankton neighbors. Possession of MAAs, which we believe can be inferred from strong UV-absorption signals in phytoplankton absorption coefficients, can be used as a flag for potential HAB outbreak. The goal of this project was to develop a solar simulating UV-visible incubator to grow HAB dinoflagellates, to begin MAA analysis of samples collected on global cruises, and to carry out initial experiments on HAB dinoflagellate species in pure culture. Our scientific objectives are to quantify MAA production and spectral induction mechanisms in HAB species, to characterize spectral absorption of MAAs, and to define the ecological benefit of MAAs (i.e. photoprotection). Data collected on cruises to the global oceans will be used to parameterize phytoplankton absorption in the UV region, and this parameterization could be incorporated into existing models of seawater optical properties in the UV spectral region. Data collected in this project were used for graduate fellowship applications by Elizabeth Frame. She has been awarded an EPA STAR fellowship to continue the work initiated by this project.

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Summary

In Year 1 we designed and built a UV/PAR solar simulator/incubator, begun culturing several species of harmful bloom-forming dinoflagellates, and developed methods for identifying and quantifying mycosporine-like amino acids (MAAs) from natural and cultured phytoplankton samples. While current data indicates that the UV-signatures of these phytoplankton species are not unique enough to allow their definite identification from natural samples, we are optimistic that by understanding the production and ecological function of MAAs we will gain insight into what conditions favor HAB species, thus allowing them to out-compete other phytoplankton, leading to large, monospecific blooms which have serious economic and health impact for coastal communities. We are also confident from preliminary results of laboratory and field samples that analysis of MAAs will allow us to obtain a better parameterization of phytoplankton absorption in the UV region. Based on the research completed in this project, Elizabeth Frame submitted graduate fellowship proposals to NASA and EPA. Her EPA STAR fellowship was selected and she will continue the work started initially on this NASA grant. We thank NASA for the year of initial funding that started a new line of research for our laboratory.

UV/PAR solar simulator/incubator

Incubation design and fabrication

In many studies of the effects of UV on phytoplankton, UV exposures are increased beyond environmentally realistic levels to highlight the effects of UVR on biological systems. In order to simulate a more environmentally accurate UV exposure we have designed and constructed a UV/PAR solar simulator/incubator system that mimics the solar spectrum with respect to the ratio of UV and visible (PAR) radiation (Figure 1). This incubator uses a 500W xenon bulb as the UV source and a 1000W tungsten/halogen bulb as the PAR source. Light from both bulbs strikes a Spectralon diffuser that homogenizes the light field before it exits the incubator box. A water-filled UV-transparent Plexiglas reservoir kept at 18°C by a recirculating temperature-controlled water bath holds the experimental culture flasks. Cultures are kept in Pyrex flasks that have 50% UV transmittance at 305nm. Environmentally appropriate UV and PAR levels can be simulated using a combination of filters and screens. Without filters, PAR measured inside a Pyrex flask centered in the water bath is 700 μEin m^{-2} sec^{-1} and UVR exposure is 1.16 W m^{-2}.

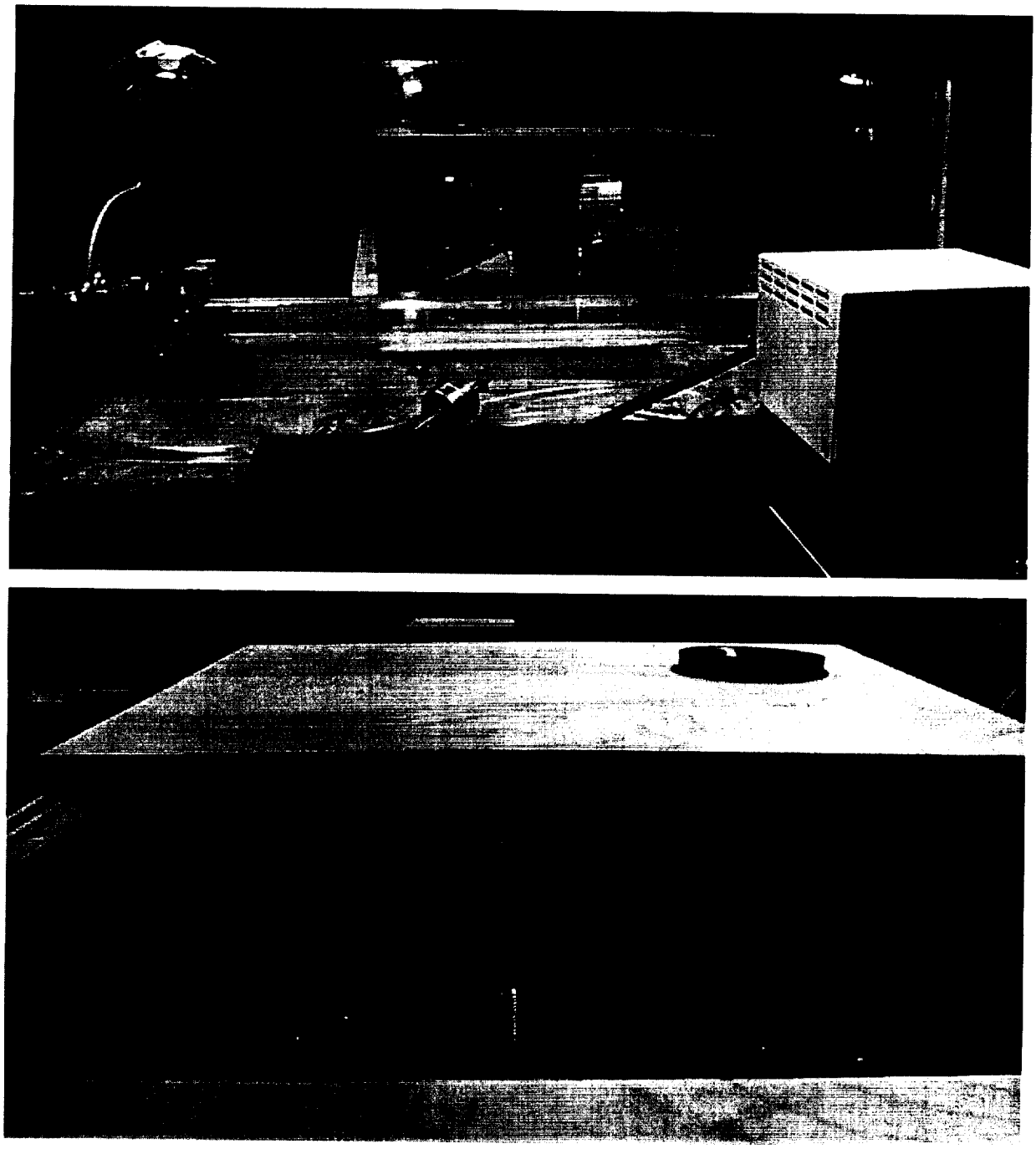

Figure 1. UV-PAR incubator system. (**A**) Front view: Solar simulator light source with mirrored front and Spectralon diffuser, UV-transparent Plexiglas tank for culture vessels, and water temperature control bath. (**B**) Internal view: Solar simulator light source with top removed showing PAR (tungsten/halogen) and UV (xenon) lamps with elliptical reflectors aimed at Spectralon diffuser.

Evolution of incubator performance

The spectral shape is fairly constant at different locations across the front of the box (Figure 2a), indicating that the Spectralon is adequately homogenizing the UV and visible light fields. However, the absolute magnitude at each location differs by as much as 20% (Figure 2b) with highest values at the center and lowest values at the far edges. While the magnitude changes across the front of the box, the ratio of UV/PAR remains fairly constant (Figure 2c), as would be expected from the similar spectral shapes between locations.

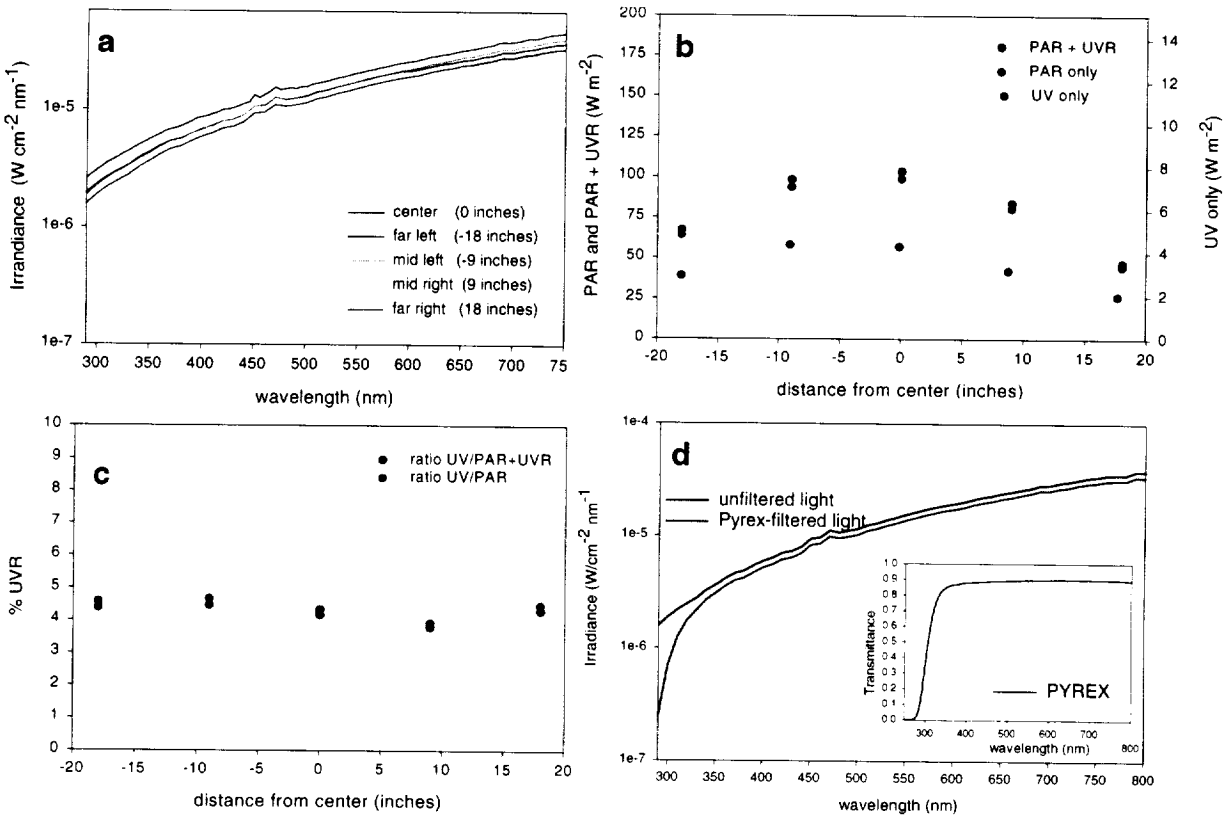

Figure 2. **(a)** Spectral shape measured at 5 locations along the front of the UV/PAR simulator (distance from center indicated in parentheses). **(b)** Flux of PAR + UVR, PAR only, and UV only at the five locations indicated in (a) (*Note different scale for UV-only values). **(c)** Ratio of UV to total (UV+PAR) and PAR-only at the same five locations in parts (a) and (b). **(d)** Spectral distribution of the incubator illumination (red) and the convolution with Pyrex culture vessel transmittance (blue). Experimental cultures are grown in Pyrex vessels to exclude short wavelength UVB flux to achieve a spectrum that is similar to natural fluxes in the environment.

HAB culturing

We have obtained and successfully cultivated several strains of bloom-forming dinoflagellates. These include *Alexandrium tamarense* (toxic and non-toxic strains, as well as one axenic, non-toxic strain), *Gymnodinium breve* (toxic strain), *Gymnodinium sanguineum* (non-toxic), *Lingulodinium polyedrum* (non-toxic), and *Prorocentrum micans* (non-toxic). MAA production has been reported in many of these species, with different species producing different combinations of MAAs.

Identification and quantification of MAAs

We have slightly modified previously reported HPLC methods for identifying MAAs from phytoplankton samples in an effort to optimize the procedure for analysis of mixed–species field samples. We have also been provided with a set of secondary standards (calibrated against primary standards from the Australian Institute of Marine Sciences) from Dr. Michael Lesser, which allows us to identify eight MAAs and quantify six of them. Identification of the remaining types of MAAs can be tentatively made from retention times and peak absorption values reported in the literature.

Response to *A. tamarense* of UV/PAR exposure

The UV/PAR simulator/incubator set-up has been run for up to 12 hours at a time and the fans and circulating water bath enable us to keep temperature of the cultures in the reservoir at a constant 18°C so as not to subject the phytoplankton to temperature stress. The simulator can be run with PAR+UVR or PAR or UVR only. Cultures of the toxic bloom-forming dinoflagellate *Alexandrium tamarense* have been exposed to PAR-only and PAR+UVR treatments using the incubator system and a time series of spectral absorption coefficients was determined. Results of this preliminary experiment are shown in Figure 3. Absorption in the UV range increased over the course of the incubation for both PAR-only and PAR-UVR treatments, but the increase is much more dramatic when UVR is present. The increase in UV-absorption is most likely due to increase in the cells' MAA-content, production of which has been shown to be stimulated by UVR or high levels of PAR.

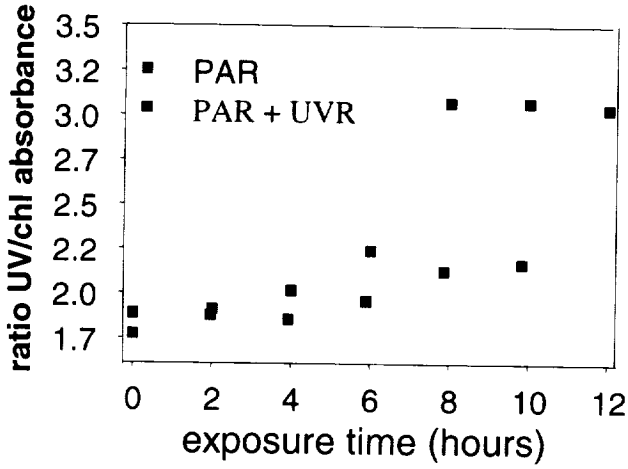

Figure 3. Chlorophyll specific UV-absorbance for *Alexandrium tamarense* exposed to PAR only or PAR-UVR.

Global ocean samples

We have collected a global data set consisting of MAA concentration, spectral absorption, UV-visible reflectance, and diffuse attenuation coefficients taken in equatorial (Indian Ocean), sub tropical (California Current, Sea of Japan), and polar (Ross Sea) waters. In all these environments very high UV absorption is observed in a considerable number of near-surface samples (Figure 4). This UV-absorption peak can be several times greater than that of chlorophyll (Figure 4 a, and b).

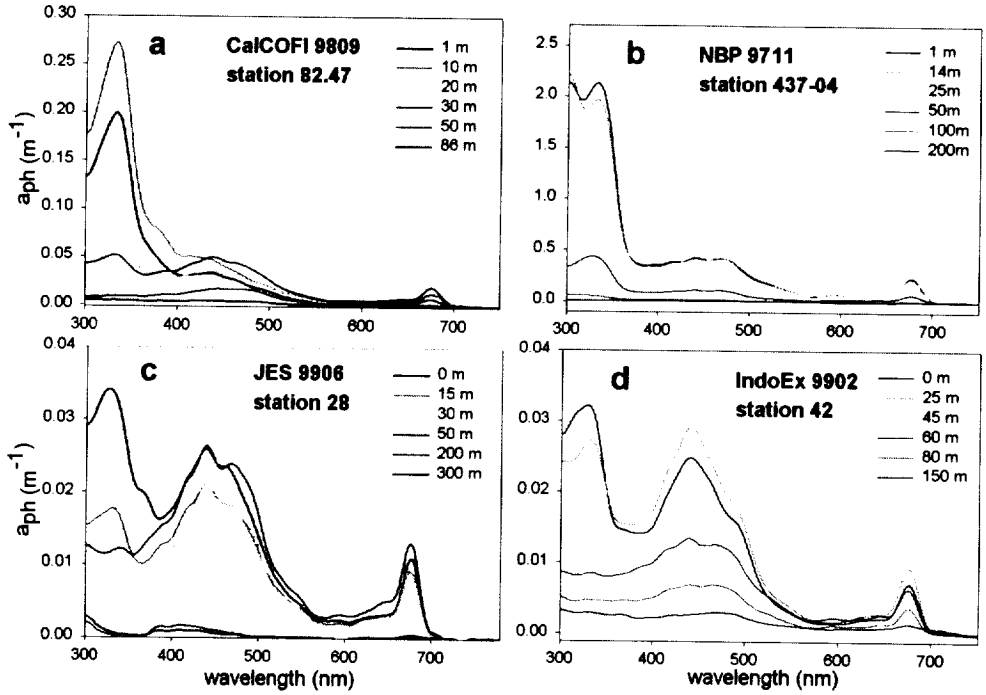

Figure 4. Spectral absorption coefficients from 300-750nm at six depths for stations in (**a**) California Current, (**b**) Ross Sea, (**c**) Japan East Sea, and (**d**) Indian Ocean. A peak in the UV range around 325nm that decreases with increasing depth is evident at all four stations.

Relation between MAAs and UV absorption

High absorption in the UV-range seen in figure 4 is positively correlated with total MAA content in samples analyzed thus far (Figure 5). The relation between MAA content and peak UV absorption appears to be linear but should be asymptotic at very high MAA concentration due to packaging effects (Morel and Bricaud, 1988; Mitchell and Keifer 1988). Analysis of this global data set of MAA abundance, absorption coefficients, and other optical parameters will enable us to characterize their relationship to determine if and how they vary between waters of low, mid, and high latitudes. This information, combined with data detailing UV effects on physiological state (which will be obtained from the laboratory experiments previously described in the proposal), will be used to parameterize phytoplankton absorption in the UV region. This parameterization will improve models of seawater optical properties in the UV spectral region such as that of Vasilkov et al., (Submitted).

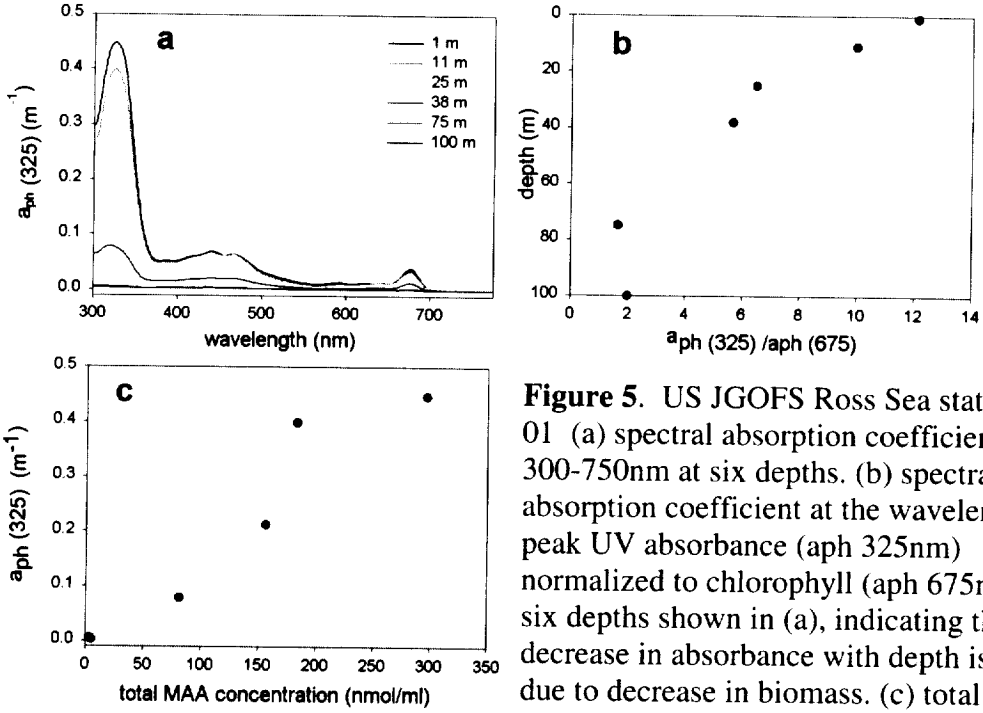

Figure 5. US JGOFS Ross Sea station 422-01 (a) spectral absorption coefficients from 300-750nm at six depths. (b) spectral absorption coefficient at the wavelength of peak UV absorbance (aph 325nm) normalized to chlorophyll (aph 675nm) at the six depths shown in (a), indicating that the decrease in absorbance with depth is not just due to decrease in biomass. (c) total MAA concentration from the six depths in (a) plotted vs. the corresponding values of aph (325nm) showing a positive, nearly linear correlation.

Future directions

Based on the research summarize above, Elizabeth Frame submitted a successful application for an EPA STAR graduate student fellowship. She will continue working on the role of MAAs in photoacclimation of HAB dinoflagellates. NASA's support for the initial research is kindly acknowledged. Future work will focus on completing analysis of the global data set as well as detailed lab experiments on MAA production/function in several toxic dinoflagellate species. This data, along with the work we have described in this report, will aid our understanding of the role of MAAs in model organisms such as dinoflagellates. Our goal is to improve predictions on the affects of changes in UV flux on phytoplankton communities. Our results on HABs will contribute to improved predictions of outbreaks of harmful, bloom-forming species. In addition, knowledge of how MAAs relate to optical properties will allow us to make more accurate parameterization of absorption by phytoplankton in the UV region. This knowledge will improve the accuracy of models, which describe UV penetration in the upper ocean and serve as the basis for UV spectroscopic methods for monitoring HABs.

References

Mitchell, B.G. & Kiefer, D.A. (1988). Variability in pigment specific particulate fluorescence and absorption spectra in the northeastern Pacific Ocean. *Deep-Sea Res.-I* **35**, 665-689

Morel, A. & Bricaud, A. (1981). Theoretical results concerning light absorption in a discrete medium, and applications to specific absorption of phytoplankton. *Deep-Sea Res.-I* **28**, 1,375-1,393

Vasilkov, A., Krotkov, N., Herman, J., McClain, C., Arrigo, K.R. & Robinson, W. Global mapping of underwater UV irradiances and DNA-weighted exposures using TOMS and SeaWiFS data products, *J.Geophys.Res.* (in press)

Appendix 1. Graduate Fellowship Proposal EPA STAR Program

PRE-APPLICATION FOR GRADUATE FELLOWSHIP

1a. Personal data:

Frame, Elizabeth R.
1129 Torrey Pines Rd. #12
La Jolla, CA 92037

Home phone: (858) 459-7704
Work phone: (858) 534-8947
Email: eframe@ucsd.edu
Fax: (858) 534-2997

White female

1b. Citizenship status: US citizen, born in Oak Ridge, Tennessee

2. Degree sought: PhD, expected date 6/03

3. Pre-application category: continuing doctoral

4a. Field of specialization code: D1

4b. Name of specialization: Oceanography

4c. Title: The role of mycosporine-like amino acids (MAAs) in harmful bloom-forming dinoflagellates: Effects of ultraviolet radiation, vertical mixing, and nutrient availability

5a. Current school and department: University of California San Diego, Scripps Institution of Oceanography, Biological Oceanography curricular group

5b. Name and address of graduate advisor:

B. Greg Mitchell
Associate Research Biologist/Lecturer
Scripps Institution of Oceanography, UCSD
8602 La Jolla Shores Dr
Sverdrup Hall 2240
La Jolla, CA 92093-0218

Phone: (858) 534-8947
Fax: (858) 534-2997
Email: gmitchell@ucsd.edu

6. Transcript information:

Institution: University of California San Diego – Scripps Institution of Oceanography
Current GPA: 3.75

year	course #	title	credits	grade
1999	SIO 209	Spec. Topic: Ocean Color Remote Sensing	4	A
	SIO 285	Physical Biological Interactions	4	A
1998	SIO 260	Marine Chemistry	4	B+
	SIO 282	Phytoplankton Diversity	4	A
	SIO 287A	Marine Microbial Ecology	4	A
	SIO 209	Spec. Topic: Ocean Optics	4	S
	SIO 240	Marine Geology	4	A
	SIO 271	Marine Zooplankton	4	A-
	SIO 296	Spec. Topic: Ecol. Evol. Genet. of Marine Org.	2	S
	SIO 278	Prob. Bio. Ocean: Harmful Algal Blooms	2	S
	SIO 296	Spec. Topic: Det. of Microb. & Activ. in Environ.	2	S
1997	SIO 210	Physical Oceanography	4	B+
	SIO 270	Pelagic Ecology	4	A-
	SIO 279	Spec. Topic: El Nino	1	S
	SIO 280	Biological Oceanography	4	A

Institution: University of Washington
B.S. degree in Biology: Cell and Molecular Biology awarded 12/96 (with college honors)
B.S. degree in Biochemistry awarded 12/96
GPA: 3.45

year	course #	title	credits		grade*
1997	BIOL 472	Principles of Ecology	5	3.4	B+
	BOT 527	Plant Molecular Systematics and Evolution	1	CR	CR
1996	MATH 351	Quantitative Methods	3	3.3	B+
	MICRO 441	Intro Immunology	4	3.4	B+
	ZOOL 470	Techniques for Mathematical Biology	3	3.2	B+
	QSCI 381	Intro Probability and Statistics	5	3.5	A-
	CHEM 437	DNA & Protein Chemistry	3	2.8	B
	CLAS 205	Bioscientific Vocab	3	CR	CR
	GENET 372	Gene Function	5	3.4	B+
	PSYCH 322	Intro Drugs and Behavior	3	4.0	A
	ZOOL 490	Undergrad seminar	3	CR	CR
	OCEAN 499	Undergrad Research	3	4.0	A
	RUSS 322	Lit and Culture 1700-1900 (honors)	5	3.7	A-
1995	BIOL 521	Advanced Cytology	1	CR	CR
	CHEM 321	Quantitative Analysis	5	2.8	B-
	RUSS 321	Lit and Culture to 1700 (honors)	5	3.9	A-

6. Transcript Information continued:

Institution: University of Washington

year	course #	title	credits	grade	
1995	ZOOL 484	Animal Physiology	3	3.7	A-
	BIOC 426	Biochemistry Lab	3	3.8	A-
	BIOC 442	Biochemistry	4	2.9	B-
	CHEM 419	Bioinorganic Chemistry	3	3.5	A-
	CHEM 436	Enzymes and Natural Products	3	2.8	B-1
	H A&S 350	Honors Seminar	2	CR	CR
	ZOOL 448	Concepts of Nervous System Function	3	3.6	A-
	BIOC 441	Biochemistry	4	2.4	C+
	BIOL 401	Cell Biology	5	2.9	B
	CHEM 352	Physical Chemistry for Biologists	4	2.8	B-
	ZOOL 409	Sociobiology	5	4.0	A
1994	BIOC 440	Biochemistry	4	3.0	B
	CHEM 416	Transition Metals	3	3.0	B
	ZOOL 403	Comparative Vertebrate Histology	5	2.2	C+
	ZOOL 433	Invertebrate Zoology	5	3.8	A-
	BIOL 203	Introductory Biology	5	3.1	B
	CHEM 455	Physical Chemistry	3	3.0	B
	GENET 371	Intro Genetics	5	3.9	A
	PSYCH 421	Neural Basis of Behavior	5	4.0	A
	CHEM 337	Organic Chemistry (honors)	4	3.2	B+
	CHEM 347	Organic Chemistry Lab (honors)	3	3.6	A-
	H A&S 263	World Civilization III (honors)	5	3.8	A-
	PHYS 123	Physics – Waves (honors)	4	3.2	B+
	PHYS 133	Experimental Physics – waves	1	3.2	B+
	CHEM 336	Organic Chemistry (honors)	4	2.5	B-
	CHEM 347	Organic Chemistry Lab (honors)	3	3.4	B+
	H A&S 252	Western Civilization II (honors)	5	3.8	A-
	PHYS 122	Physics – Electromag. and Oscill. Motion (hon)	4	3.2	B+
	PHYS 132	Experimental Physics – Electromag. and Oscill.	1	3.2	B+
1993	BIOL 202	Introductory Biology	5	4.0	A
	CHEM 355	Organic Chemistry (honors)	4	2.3	C+
	H A&S 261	World Civilization I (honors)	5	3.7	A-
	PHYS 121	Physics – Mechanics (honors)	4	2.9	B
	PHYS 131	Experimental Physics – Mechanics	1	2.9	B
	BIOL 201	Introductory Biology	5	4.0	A
	CHEM 164	Intro Inorganic and Environmental Chemistry	5	3.9	A
	H A&S 253	Western Civilization III (honors)	5	3.9	A
	RUSS 203	Second Year Russian	5	3.1	B
	CHEM 155	General Chemistry (honors)	4	3.2	B+

6. Transcript Information continued:

Institution: University of Washington

year	course #	title	credits	grade	
1993	CHEM 157	General Chemistry Lab (honors)	3	4.0	A
	MATH 126	Calculus and Analytic Geometry III	5	3.2	B+
	RUSS 202	Second Year Russian	5	3.4	B+
1992	CHEM 145	General Chemistry (honors)	4	4.0	A
	H A&S 251	Western Civilization I (honors)	5	3.8	A-
	MATH 125	Calculus and Analytic Geometry II	5	3.4	B+
	RUSS 201	Second Year Russian	5	3.9	A

*the University of Washington uses a numerical grading system (4.0 scale). I have converted the numerical grade to the corresponding letter grade using the official table in the UW course catalog and student handbook.

GRE General Exam Scores (taken 4/95)
Verbal: 670 Quantitative: 750 Analytical: 660

7a. Experience:

1997-1998 Studied the changes in abundance, distribution, and pigment content of the cyanobacteria *Synechococcus* sp.in the California Current during the '97 El Nino. The results of this work were presented as a poster at the 1998 Ocean Sciences Meeting.

1995-1997 Worked with Dr. Evelyn Lessard at the University of Washington School of Oceanography on developing rRNA-targeted oligonucleotide probes to determine growth rates in heterotrophic marine protists. Growth rates of marine protists are important, as these organisms are a major link between lower levels of the food web (phytoplankton and bacteria) and higher levels (zooplankton and larval fish). I learned the use of oligonucleotide probes, epifluorescent microscopy, and image-analysis programs while working on this project.

December-January 1995-6 Participated in a six-week research cruise aboard the *RV/ Nathaniel B. Palmer* to the Ross Sea (Antarctica) with Dr. Lessard, studying grazing of heterotrophic protists during a *Phaeocystis* bloom in the Ross Sea polynya. On this cruise I assisted with dilution experiments to measure grazing rates of heterotrophic protists, measured chlorophyll to determine growth of phytoplankton, and counted bacteria to determine their growth and abundance, thus covering growth and grazing of lower trophic levels in the marine environment. Results of this work were presented by Dr. Lessard at the 1998 Ocean Sciences Meeting.

1993 Worked with Dr. Robin Wright at the University of Washington Zoology Department to clone a copper-induced promoter into the HMGCo-A reductase gene. HMGCo-A reductase is the enzyme that regulates the rate-limiting step in cholesterol synthesis. An understanding of how this gene works and is regulated could have consequences for treatment of high cholesterol and heart disease. For this work I learned basic techniques of DNA transformation and ligation.

Summer 1991 Participated in an eight week NSF Science program for high school students, studying transmembrane signal transduction in *Amoeba proteus* with Dr. Robert Prusch at Gonzaga University, Department of Biology. My project consisted of performing experiments to determine if G-proteins are involved in signal transduction in *A. proteus*. After spending many hours watching the cells to determine "normal" feeding behavior I treated them with various G-protein inhibitors and observed their ability to detect and capture prey items. I wrote up the results of these experiments and was selected as a semifinalist for the national Westinghouse Science Talent Search, a competition for science papers written by high school students.

1990 Participated in a summer science program at Washington State University in the microbiology lab of Dr. John Paznokas exploring the use of the fungus *Mucor racemosus* in bioremediation of sites contaminated with heavy metals. The fungus *M. racemosus* shows an ability to bind copper and in this way remove it from the environment. My project was to design simple experiments to train this fungus to withstand high copper levels, which I did by gradually increasing the amount of copper in their agar substrate.

7b. Publications: NA

8. Statement of Objectives.

I have chosen a career in oceanography due to its global importance and multidisciplinary nature. Covering over 70% of the Earth's surface, oceans control climate, provide a home for a vast array of life, and are an important resource to humans, providing protein-rich foods and many other products. Oceans are a fundamental part of the global ecosystem, yet many basic questions about life in the ocean remain unanswered or have only recently begun to be addressed. To adequately address any question about biology in the ocean an understanding of the physics and chemistry that govern the marine environment is essential. This is an exciting intellectual challenge, requiring integration of concepts and tools from many different fields. As an undergraduate I laid the foundation for such a career. I majored in biochemistry and cell biology, but choose to take additional courses in chemistry, math, and physics. I discovered many useful tools and concepts in these courses that helped me better understand biology. For example, I found biological systems with their complex rates and fluxes much easier to understand once I had been introduced to differential equations. I am attracted to multidisciplinary topics that require the synthesis of different intellectual skills and concepts.

My interest in science and the environment developed early. In high school I participated in an NSF program introducing students to academic research. The project I was assigned to involved studying the use of a fungus capable of binding heavy metals, removing them from contaminated mining sites. The application of biology to solve real-world problems was very exciting to me. As an undergraduate I had an opportunity to apply knowledge of molecular and cell biology to an environmentally relevant question – the determination of *in situ* growth rates in heterotrophic marine protists. This work was very rewarding because I used a relatively new technique developed in molecular and cell biology to address a basic ecological question that had long been difficult to answer with traditional techniques. This project first directed my attention towards oceanography as a field where opportunities abound for integrating concepts and techniques from diverse disciplines to address fundamental questions.

As a graduate student at Scripps I am constantly reminded that knowledge of biology alone is not enough to make a successful biological oceanographer. A seminar I took last fall on harmful algal blooms highlights this point. Each week we discussed a different aspect of blooms and bloom formation, covering biology, physics, chemistry, biochemistry, and microbiology. I had already developed an interest in UV photoprotection in phytoplankton and saw bloom-forming species, which spend daylight hours near the surface where UV flux is highest, as perfect systems in which to study this phenomenon. Last spring a course on physical/biological interactions spurred my interest even more, providing me with tools to understand physical forcings (mixing and turbulence) that can affect a cell's UV exposure. These ideas form the basis of my proposal and my graduate thesis. My research goal is to determine how a class of UV-absorbing compounds, MAAs, affect bloom formation in harmful dinoflagellates and how this knowledge can be used to predict when and where blooms will occur. I will accomplish this by combining tools and concepts from biochemistry, biology, and oceanography.

After receiving my Ph.D. I plan to continue researching oceanographic questions of direct importance to humans using an approach where I integrate controlled laboratory studies with field work, designing lab studies to be environmentally relevant and ensuring that interpretations of field observations have a solid foundation in controlled laboratory work. My planned thesis work, combined with strong courses available at Scripps covering a variety of oceanographic disciplines, will give me practical training and intellectual preparation for such a career.

9. Narrative Statement

A. Goal of Research

Changes in stratospheric ozone levels over the last two decades have focused attention on ultraviolet radiation (UVR) and its effects on the environment. Decreases in ozone concentration allow a greater percentage of harmful UVB radiation to reach the earth's surface (Crutzen, 1992). In the past two decades there has been an observed increase in the number and magnitude of blooms of harmful algal species, primarily dinoflagellates (Smayda, 1990; Anderson, 1995). Bloom-forming species typically spend long periods of time at the surface, where they are exposed to high levels of UVR and visible radiation. UVR has been shown to affect phytoplankton in many ways including inhibition of photosynthesis, DNA damage, changes in membrane integrity, motility, nutrient uptake, cell division, and many other physiological properties. Surface bloom-formers must possess mechanisms to prevent or mitigate this damage.

One mechanism for preventing UV damage is the production of compounds that absorb the energy from harmful UV rays. One class of UV-absorbing compounds is mycosporine amino acids (MAAs). Over 20 types of MAAs, with peak absorption maxima ranging from 310 –360 nm, have been identified in marine organisms, including dinoflagellates (Karentz et al, 1991a; Carreto et al, 1990). These compounds confer protection from UVR to sea urchin eggs (Adams and Shick, 1996) but their photoprotective role in phytoplankton though widely speculated, has yet to be definitively proven. A rapid response to environmental variables that change on time scales of hours to days, such as UV exposure during mixing conditions, is necessary for MAAs to be an effective sunscreen. On much longer time scales anthropogenic-affected environmental variables such as average daily UV irradiance and coastal nutrient influxes may influence MAA production in phytoplankton. Thus an understanding of how MAA production is regulated in a dynamic environment will be instrumental to better understand what conditions lead to bloom formation and how anthropogenic effects on ozone levels and coastal eutrophication may influence surface bloom-forming phytoplankton.

Hypothesis: MAAs play a role in some dinoflagellates' ability to form near-surface blooms. To understand this role, four focused hypotheses will be addressed:

1. **HAB species *Gymnodinium breve* and *Alexandrium tamarense* produce MAAs when exposed to high levels of UV or visible radiation; levels and production rate of MAAs depend on UVR exposure.**
2. **MAAs act as photoprotective compounds in *G. breve* and *A. tamarense*.**
3. **Nitrogen status of cells will affect MAA production.**
4. **Vertical mixing rates determine the effectiveness of MAA production for UV photoprotection in the environment.**

B. Rationale

Gymnodinium breve is a dinoflagellate species that blooms frequently in the Gulf of Mexico. Aerosols released from these cells cause respiratory distress in humans and other animals in coastal areas (Feinstein 1955). *G. breve* is positively phototactic and spends long periods of time at the surface during the day, moving into deeper, nutrient-replete waters at night (Holligan, 1985). No studies have addressed the MAA composition of *G. breve* but a close relative, *G. sanguineum*, has been shown to possess MAAs as possible photoprotective compounds (Neale et al, 1998). *Alexandrium tamarense* is another bloom-forming dinoflagellate, capable of producing toxins that can be concentrated in the tissues of mussels, clams, oysters and other filter feeding organisms. When humans or mammals eat these shellfish they can contract paralytic shellfish poisoning (PSP), symptoms of which include nausea, vomiting, and death. *Alexandrium* blooms occur every

year in coastal waters of the northeastern US and Canada (Anderson et al, 1982) and have been reported on the Pacific coast of North America as well (Horner et al, 1997). When blooms are reported or suspected shellfish fisheries are closed, causing a severe economic impact on coastal communities. *A. tamarense* vertically migrates (MacIntyre et al, 1997), spending daylight hours near the surface where it can form dense blooms (Sakshaug and Jenson, 1971). This is another species that may benefit from possession of sunscreen compounds such as MAAs.

MAAs may confer a competitive advantage to these cells, allowing them to remain near the surface while photosynthesizing during the day, thus shading out phytoplankton competitors. This, coupled with their ability to vertically migrate away from nutrient-depleted surface waters into deeper, nutrient-replete waters at night, may be one attribute that allows them to form massive, monospecific blooms (Carreto, 1990; Vernet et al, 1989).

General knowledge of phytoplankton response to increases in UVR is important not just in the Antarctic under the ozone hole, but in mid latitudes as well where cells already receive very high doses of UVR. An increase in UV dose to these systems, which are already operating at high UV levels, could have a significant impact. Species that can adapt to these conditions will have a distinct advantage over competitors that cannot (Davidson et al, 1996). Studies of four Antarctic diatom species (Helbling et al, 1996) showed that the species which produced more UV-absorbing compounds (MAAs) had lower inhibition of photosynthesis than species that had little or no production of UV-absorbing compounds. Vernet et al (1994) reported that Antarctic species with high pigment-specific UV-absorption showed a smaller decrease in photoinhibition when UVR was removed than species with a low pigment-specific UV-absorption, indicating that the UV absorbing compounds have a photoprotective role. If dinoflagellates are able to regulate the production of MAAs, coordinating it with exposure, they may be able to dominate areas with high UV-flux.

Previous research on phytoplankton and MAAs has focused heavily on Antarctic species. Few recent studies have directly addressed the question of MAAs in HAB species. The effects of high irradiance on MAA production in *Alexandrium tamarense* (previously *A. excavatum*) were studied by Carreto et al (1990). *A. tamarense* was transferred from low to high light conditions, inducing rapid production of UV-absorbing compounds identified as MAAs. Results of Neale et al (1998) suggest that MAAs may provide UV-photoprotection in *G. sanguineum*, another bloom-forming dinoflagellate. MAA production was induced with high levels of visible radiation and MAA photoprotection was evaluated under UV radiation. Vernet et al (1989) and Vernet and Whitehead (1996) suggest that UV-absorbing compounds in the bloom-forming dinoflagellates *Lingulodinium polyedrum* and *Prorocentrum micans* have a photoprotective effect. In contrast, Lesser (1996), did not find that UV-absorbing compounds prevented UV-induced photodamage in *P. micans*. However, Lesser's study consisted of a 21 day incubation in which samples were only taken at the end points. This highlights a trend in studies of MAAs: Cell cultures are incubated for extended periods (several days to several weeks) and sampled rather infrequently (often every 1-3 days). The rationale behind this approach is to allow cells to become fully acclimated to a given set of light conditions so that physiological characteristics, such as growth rate, have reached approximate steady state. However, in natural environments cells are not exposed to the same light conditions for the entire day, much less several weeks. One of the few studies (Carreto et al, 1990) which involved sampling on small time scales (every three hours) found that upon transfer from low to high light, UV-absorption in *A. tamarense* increased strongly in the first three hours, then declined slightly in the next three hours. This indicates the need for frequent sampling.

Incubation times and sampling rates need to be determined which accurately reflect the changing light conditions a cell would encounter in the environment.

MAAs are produced from 3-dehyroquinate (DHQ) an intermediate in the shikimate pathway, the biochemical pathway by which aromatic amino acids are produced (Bentley 1990). MAAs contain an amino acid-like component and an aminocyclohexenimine ring (Bandaranayake, 1998), both require nitrogen. Agricultural and urban runoff has lead to increased inputs of nitrogen into coastal waters (Paerl, 1997), providing phytoplankton with an additional source of nitrogen; availability of which normally limits their growth. Increasing the nitrogen available to a MAA-producing species may allow it to manufacture MAAs at a greater rate and/or concentration than would be possible under limiting conditions when available nitrogen may be allocated for protein production and growth rather than UV photoprotection.

Blooms of dinoflagellates are often associated with calm conditions and a stratified water column. It is often suggested that dinoflagellates are particularly poorly adapted to turbulent water conditions (Margalef, 1978), possibly due to high sensitivity of dinoflagellates to fluid motion (Pollinger and Zemel, 1981; Thomas and Gibson, 1990; Berdalet, 1992). Complementing this idea is the suggestion that dinoflagellates are especially well adapted to stratified conditions (Margalef, 1978; Estrada and Berdalet, 1997). In calm conditions cells may become trapped at the surface due to strong pycnoclines, subjected to high UVR and PAR fluxes. Under such conditions the ability to produce photoprotective compounds is expected to afford significant benefit to algal cells. In contrast, under conditions of active water column mixing the light levels a cell experiences may change due to advection faster than the synthesis of photoprotective compounds (MAAs) can be regulated, thus making them inefficient sunscreens. Knowledge of the time scales at which MAAs are synthesized and how MAA levels relate to mixing in the environment may lead to greater ability to predict what conditions will be favorable to bloom development.

C. Approach and expected results

To test the hypothesis that MAAs influence harmful dinoflagellates' ability to form near-surface blooms I will address four specific hypotheses using two species which are responsible for harmful blooms along US shores, *Alexandrium tamarense* and *Gymnodinium breve*.

Hypothesis 1: The HAB species *Gymnodinium breve* and *Alexandrium tamarense* produce MAAs when exposed to high levels of UV or visible radiation

Preliminary experiments show *A. tamarense* is capable of producing MAAs. I have grown cultures of *A. tamarense* (CCMP115) under fluorescent lights at high and low levels of PAR. Cultures grown at high light have noticeable UV absorption while those grown at low light have very little. HPLC analysis (after method described by Dunlap and Chalker, 1986, with minor modifications) show that the high light culture produced UV absorbing compounds which appear to be the MAAs palythine, shinorine, asterina 330, and porphyra 334. I will obtain *G. breve* cultures from CCMP and expose them to high levels of PAR and determine, via HPLC analysis, if they produce MAAs.

To test the cells' response to different levels of UVR I have constructed a UV/VIS incubator that mimics the solar spectrum in respect to the ratio of UV and visible radiation. This incubator uses a 150W xenon bulb as the UV source and a 500W tungsten bulb as the visible source. Light from both bulbs goes through a diffuser that homogenizes the light field before it reaches the dinoflagellate cultures that are kept in a temperature controlled water bath/incubator made of UV transparent Plexiglas. Cultures are kept in Pyrex flasks that have 50% UV transmittance at 305nm. Environmentally appropriate UV and PAR levels will be simulated using a combination of filters and screens. Flasks will be sampled frequently for the following variables during the experiment

to get an accurate time resolution for MAA induction and an assessment of cell condition: MAAs will be identified and quantified using HPLC (Dunlap and Chalker, 1986 with minor modifications). Secondary standards that have been calibrated from primary standards will be used to determine the identity and quantity of MAAs produced. Total protein will be measured (BCA protein assay kit) at each sampling as an indicator of biomass for normalizing MAA content. Samples will also be taken for cell growth and sizing since it has been reported that UVR can inhibit cell division, leading to larger cells (Karentz 1991b). Photosynthetic energy conversion efficiency, an indicator of PSII health, will be monitored using pulse amplitude modulated fluorometry (PAM). PSII function will also be assessed by following turnover of the PSII reaction center protein, D1, using pulse chase radiolabeling. Other indicators of photodamage/repair will be monitored, including activities of enzymes superoxide dismutase and catalase, which protect the cell from superoxide radicals. Several experiments will be conducted, each with a different level of UVR, to determine if UV dose affects MAA production rates. I expect MAA production to occur at a faster rate in experiments with higher UV dose.

Hypothesis 2: MAAs act as photoprotective compounds in *G. breve* and *A. tamarense*.

Published results strongly suggest that MAAs confer UV photoprotection to dinoflagellates. However it is difficult to prove that MAAs provide protection from UVR unless cultures of one species can be produced that differ only in possession of MAAs. This has not been possible to date. Controls for most published experiments consist of cell cultures which are not exposed to UVR or cultures which have not been acclimated to UV conditions and do not produce as many MAAs as the UV-acclimated cells used in the experiment. This approach is problematic due to importance of light history in photoacclimation and photorepair; different wavelengths of light interact in various damage/repair mechanisms, making it difficult to draw firm conclusions from experiments such as those described above. To obtain non-MAA-producing cells I will block MAA production by using a cyclohexenyl or cyclohexylidene inhibitor (Montchamp and Frost, 1997) of 3-dehydroquinate synthase (DHQ synthase), the enzyme that catalyzes the step by which 3-deoxy-D-arabino-heptulosonate 7-phosphate (DHAP) is converted to 3-dehydroquinate (DHQ). Blocking DHQ production will inhibit the formation of compounds which are made downstream, such as aromatic amino acids, as well as MAAs. Careful controls must be designed to examine the pleiotropic effects of DHQ synthase inhibition. To accomplish this cells with and without inhibitor will be incubated with and without UVR and damage to components of interest (PSII function) will be measured. Side-effects of the inhibitor will only be a problem if damage from the inhibitor is greater than that from UVR exposure. If this is the case it may be possible to provide these cells with media that contains the aromatic amino acids it will not be able to synthesize. Once control cells are made these cells could then be exposed to exactly the same light conditions as the experimental cells, allowing for a more definitive proof of the role of MAAs as photoprotective compounds. Experiments would consist of exposing MAA-blocked cells and normal cells to UVR and monitoring basic physiological parameters such as photosynthesis. In this manner I will collect data that will indicate how much UVR is required to inhibit basic cell processes like photosynthesis and PSII function and how well MAAs prevent this damage. I expect cells that cannot produce MAAs to suffer greater damage than cells that can produce MAAs.

Hypothesis 3: Nitrogen status of cell cultures will affect MAA production.

MAAs are the product of a precursor in the shikimate pathway, branching off the main pathway at DHQ (Bentley, 1990). DHQ does not contain any nitrogen, but most MAAs have two nitrogenous groups. When nitrogen (N) is scarce, production of MAAs may be decreased. I will use the incubator described earlier to conduct experiments with N-replete and N-limited cultures.

N-limited cultures will have the same media as N-replete cultures, except for a smaller concentration of N, which will induce N stress more rapidly. Both groups will be exposed to UV levels that were determined in earlier to be detrimental to cells without MAA protection. Measurements will be made every hour for MAA content, total protein, PAM assessment of PSII function, and turnover of D1 protein. Since nitrogen limitation will affect other cellular functions, total proteins and turnover of photosynthetic proteins such as D1 will be monitored in addition to MAAs. Insight into the importance of MAAs' role can be inferred from allocation of nitrogen. Experiments will also be conducted with phosphate limitation (MAAs contain no phosphate) to test whether any effects seen are N-specific or a general nutrient-stress response.

Hypothesis 4: Vertical mixing rates determine the effectiveness of MAA production for UV photoprotection in the environment.

Induction time scales from the previous section will be compared to vertical mixing time scales determined by a simple model (Lande and Wood, 1987) which predicts the time it takes for a cell to pass through the mixed layer and arrive at a certain depth in the thermocline below which UV exposure is not harmful. The time given by this model is an estimate of UV exposure, not an absolute residence time. The model requires input for a vertical eddy diffusivity parameter that can be estimated from wind speed via frictional velocity. I have a large number a MAA samples and wind speed measurements from cruises in the California Current that can be used to investigate the relationship between mixing and MAA production. *G. breve* and *A. tamarense* do not bloom in the California Current but this system is the subject of a large, long-term data set and will provide enough data to construct a model which should be applicable in areas suffering from blooms of these harmful species. Preliminary data indicates cells that spend more time in the zone where UV damage is likely to occur have higher absorption in the UV range than cells which sink out of the UV-damage zone more rapidly. UV-absorption was used as a proxy for MAA content in these preliminary analyses. It must be noted that the severity of UVR exposure will not be constant over the entire depth. Shorter, more damaging wavelengths of UVR are rapidly attenuated so rays near the surface have much more damage potential than those lower in the water column. UVR data at two wavelengths, 340 and 380 nm, are available for all cruises. From this data an estimate of attenuation coefficient for UVR can be made and incorporated into the model.

Knowledge of MAA-induction time scales and mixing conditions may allow improved ability to predict when blooms of harmful species are likely to occur. This could aid monitoring programs, alerting officials when conditions are indicative of bloom-formation. Remote measurement could be made of temperature changes (proxy for mixing) and UV absorption (proxy for MAA abundance) via moored buoys. This could direct sampling to areas in which blooms are likely to occur.

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