ULTRAVIOLET RADIATION AND CORAL REEFS

EDITED BY:

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HAWAI'I INSTITUTE OF MARINE BIOLOGY

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Introduction

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It has been many years since solar ultraviolet radiation was clearly identified as an important ecological factor on coral reefs (Jokiel, 1980), so it seemed timely to organize a major multi-disciplinary project designed to evaluate the state of the art, conduct research, train new researchers in the field and evaluate techniques and methods of measurement in common use today on coral reefs. Major funding for the research and educational function was granted by the Edwin W. Pauley Foundation for an advanced research and training program at the Hawai'i Institute of Marine Biology (HIMB) entitled "Ultraviolet Radiation and Coral Reefs" that was held from June 15 to August 2, 1994. In addition, the University of Hawaii Sea Grant Program funded an international workshop entitled "Measurement of Ultraviolet Radiation in Tropical Nearshore Environments" that was held at the East-West Center (University of Hawai'i, Honolulu, Hawai'i) from August 3-5, 1994. The US-Israel Binational Science Foundation supplied additional funding for participants from Israel and supported the participation by Dr. Dave Mauzerall, our keynote speaker for the workshop.

This volume contains much of the information developed during the 1994 program. Additional research papers are still in preparation by some of the participants and will be forthcoming in various journals. A very important intangible outcome was the sharing of ideas, establishment of research links between various groups and the formulation of new research directions. One example of this was the design of an underwater video system capable of detecting visual patterns in the UV portion of the spectrum. Many fish and invertebrates have the ability to visually detect UV, so they must somehow use UV to obtain information about their environment. This totally new instrument will allow us to "see" as these fish and invertebrates "see" in the UV-range for the first time. The construction of the instrument designed during the 1994 program was recently funded by the US National Science Foundation. Although the instrument will be located at HIMB, it will be used by an international group of scientists including the workshop participants that contributed to its design.

The 1994 program addressed topics from the molecular to the ecosystem level. One can simply examine the following "Table of Contents" to gain appreciation for the wide range of UV topics covered by the participants. By the end of the program, there was a general consensus on the following major points:

- UV is an important environmental factor in shallow tropical ecosystems, influencing living systems at all levels of organization from molecular to community. The importance of UV should not be surprising, given the role of this factor in the origins and evolution of life as described in Dr. Mauzerall's plenary address.
- Although the all-pervasive influence of UV can be shown by experimental treatments of "UV present" vs. "UV absent", it is another matter to conduct experiments that evaluate the possible importance of future increases in UV resulting from anthropogenic ozone thinning. Major obstacles to progress in this area include: 1.)lack of data on spectral irradiance reaching the earth's surface at low latitudes, 2.) lack of a reliable predictive model that can provide data on future increases in spectral irradiance, and 3.) lack of biological data on possible importance of such increases. An immense expenditure of research resources will be required to gain the needed information in these areas due to the technical difficulties encountered in measurement of UV, difficulties in simulating experimental regimes of increased UV and complexities in evaluating effects of a slight UV increase on an ecosystem.

- The chief obstacle to assessment of increased UV on shallow water tropical reef communities lies in their demonstrated complexity, susceptibility and adaptability to UV. Using the terms "susceptibility" and "adaptability" appears to be a paradox. On one hand, reef organisms are living at the highest levels of UV found in the oceans, and show remarkable ability to adapt to extremely high levels of UV. Can these organisms readily adapt to levels of UV that are even higher than presently encountered at the surface of the ocean? This is probable, but has yet to be demonstrated. The UV-blocking compounds in reef corals serve as a good example of a mechanism allowing corals to adapt to a wide range of UV environments at little metabolic cost. On the other hand, UV has been shown to influence most aspects of coral metabolism and impacts all stages in the life cycles of various organisms. In addition, interaction of UV with other physical factors (e.g. temperature-UV synergism's) or processes such as UV-phototoxicity can exacerbate the intensity of other environmental stresses acting on reefs, and in these cases UV produces more damage than anticipated. Every argument as to the sensitivity of reefs to UV damage seemingly can be met with a counter argument that adaptation or acclimation will return the reef to equilibrium.

- Participants in the 1994 program came to the conclusion that UV research must always be "question-driven". This attitude was most apparent in discussions of UV-measurement instrumentation. The group advocated use of appropriate measurement techniques for the question being asked, with due regard to cost effectiveness, rather than selection for standardized instrument. Many biological questions can be resolved using simple and inexpensive actinometric techniques or dosimeters. Some questions are best asked using Robertson-Berger sunburning units or similar units. Other questions, including the monitoring of existing and future trends in solar UV irradiance reaching the surface at low latitudes will require extremely sophisticated scanning spectroradiometric monitoring systems that could be prohibitive, given present research funding levels. Again, the participants argued that first one must ask a legitimate scientific question and then specify the UV measurement technique to be employed.

- It is clear that we have only scratched the surface of basic UV-related research topics. The first demonstration of UV-phototoxicity was conducted during the 1994 program (Peachey and Crosby, this volume). Our embarrassingly small base of information on UV and coral reproduction was expanded by the studies of Gulko (this volume) and Baker (this volume). Scientists concerned with the emerging question of UV, polarization and vision in tropical marine animals point out the lack of information in this important area (Shashar, Shashar et al., this volume, Shashar et al., this volume, Losey et al., this volume). The first broad scale measures of UV attenuation in Kaneohe Bay were made during the program (Patterson, this volume). Numerous other studies in this report demonstrate the fertile research ground available for UV research in tropical marine systems, and a myriad number of research questions were raised by the particiants (e.g. Discussion at Workshop Sessions, this volume).

- The group as a whole recognized the need for research centers in tropical reef areas that would be available to all. This recommendation is being implemented. We have secured an NSF grant to purchase the underwater UV video system mentioned earlier for use by the group of international scientists involved in this work. Further, NSF has provided funds to purchase a scanning underwater UV spectroradiometer for HIMB. The data base developed in Kaneohe Bay during the session will be invaluable to researchers using our facility. The University of Hawaii recently purchased all of Coconut Island (which includes housing to be used by visiting investigators) and is building a new $8.3 million dollar laboratory with a gift from the Edwin W. Pauley Foundation. Therefore, we are well on the way of meeting this recommendation by establishing this facility as a center for UV research.

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Ultraviolet Light and the Origin of Life

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Since most of the talks at this symposium will be on the nastier aspects of UV light, I wish to put in a good word for this active radiation. I will describe how it may have provided redox reactions for the chemical origin of life on the primitive earth. I will begin by describing the surprisingly voracious appetite of living cells for energy. We will reach the not too surprising conclusion that evolution REQUIRED the use of solar energy, and that is in fact how it occurred. The argument for the photochemical origin of life is less secure, but either it did so or the early cells were vastly more simple in metabolism and reproductive mechanisms than those we know about today.

Life is a highly organized system which requires a continual flux of free energy to remain in the living state and a much greater flux of both energy and matter for reproduction. It is convenient to express this flux in units of power per area, since the biosphere is a rather thin layer on the earth, and these units are just those of photon fluxes. Table I shows that only solar energy can supply the energy flux required by modern organisms. The second largest energy flux is that of lightening, which fails to supply the energy required by slow growing yeast by two to three orders of magnitude. Broda gave a striking illustration of this energy utilization by pointing out that a hard working azotobacterium, fixing nitrogen in the aerobic atmosphere and duplicating rapidly, consumes 10^8 times as much energy as the sun emits on a per weight basis. Even a quiescent human sinks 10^5 times the solar output. Living cells are little black holes for energy.

This energy requirement can be calculated on a global basis. Given a biomass of 6×10^{17} g carbon, the area of the earth, 5×10^{18} cm^2 and the energy requirement of 10^{-3} $W(gC)^{-1}$, one reaches the same energy flux as above, 10^{-4} W cm^{-2}. This value is quite independent of details. Given a biomass turnover time of 2 - 20 years and the energy needed to fix a mole of C, 500 kJ $(mole)^{-1}$, one again obtains 10^{-4} to 10^{-5} W cm^{-2}. Based on the isotopic ratios of C, and also of S, in various rocks, Schidlowski *et al.* (1983) argue that the biomass has been constant for some 3.5 Gyr. One is forced to the conclusion that solar energy was required for evolution.

Whether the origin of life required solar energy is an open question. The early cell may have been simpler, but it was probably also less efficient, if that word can be applied to the modern power-guzzling cell. If so, much energy would be needed to assemble the self-duplicating organism. The alternative is an extremely simple, metabolically and duplicatively, organism of which we have no knowledge.

We shall reduce the problem to a yet more simple level: the origin of the semi-reduced organic molecules that are the components of all living matter. The reason that this is a problem is the failure of Urey's model of the primitive atmosphere. He believed in a cold accretion of the earth, allowing time for the metallic iron to reduce nitrogen to ammonia and carbon dioxide to methane. Miller's great experiment showed that electric discharge in such an atmosphere along with water vapor led to the copious formation of amino acids and other anthropologically desirable molecules. Unfortunately geophysicists now argue that the earth accreted rapidly enough to lose iron metal to the core very early. The primitive atmosphere was composed of nitrogen and carbon dioxide. The fact that volcanic emissions are largely CO_2 with only traces of CH_4 supports this argument. More unfortunately, but predictably, the quantitative experiments of Miller and coworkers have shown that the yield of amino acids decreases dramatically as the ratio of H to C in the presumed atmosphere decreases, becoming undetectable with CO_2 alone. Thus we have a dilemma.

What is needed is a source of reducing equivalents to make the carbon biochemically useful. It is interesting that of the five stable formal valence states of carbon:

(+4)	(+2)	(0)	(-2)	(-4)
CO_2	HCO_2H	HCHO	CH_3OH	CH_4

Table I. Energy Fluxes Requirements and Evolution.

ENERGY FLUXES	
	$W\,cm^{-2}$
ACTIVE BACTERIA	-10^{-2}
YEAST	-10^{-5}
SOLAR, VISIBLE λ	$+10^{-1}$
LIGHTNING	$+10^{-7} - 10^{-8}$

ENERGY REQUIREMENTS	
	$W\,g^{-1}$
AZOTOBACTERIA	-10
QUIET HUMAN	-10^{-2}
SUN	$+10^{-7}$ (BRODA, 1975)

LIVING CELLS ARE **BLACK HOLES** FOR ENERGY

ENERGY AND EVOLUTION

BIOMASS	6×10^{17} g C
EARTH'S AREA	$5 \times 10^{18}\,cm^2$ so 10^{-1} g C cm^{-2}
IF 10^{-3} W gC^{-1}	10^{-4} g C cm^{-2}

BIOMASS – CONSTANT BY CARBON, SULFUR ISOTOPES, 3.5 Gyr
(SCHIDLOWSKI *ET AL.*, 1983)

∴ **SOLAR ENERGY REQUIRED FOR EVOLUTION**

biology operates at the zeroth or formaldehyde level. This is also by far the most reactive of the simple chemical forms of carbon. The self-zero valence level of carbon: graphite, diamond or amorphous carbon, is chemically inert.

Enter photochemistry. A most common form of photochemical reaction is that of electron transfer. These elementary reactions reduce and oxidize the reactants and will even drive reactions thermodynamically uphill, storing some of the energy of the absorbed photon. The epitome of these reactions is photosynthesis itself.

Ultraviolet photochemistry is particularly relevant since even the simplest of molecules absorb sufficiently short wavelength UV. More to the point UV was available on the primitive earth because of the absence of oxygen and thus of ozone. Table II shows the available energy on the primitive earth. There are one to two orders of magnitude more energy in even the extreme UV (<200nm) than in the other non-photonic energy sources. The energy of such photons, 6 eV, is sufficient to break chemical bonds and oxidize or reduce any known chemical compound. In fact the abundance of such photons guarantees a short lifetime for CH_4 (10^6 yr) and NH_3 (10^2 yr). The photochemistry of the atmosphere is a very active field and the elaborate computer models of these complex systems have been extended to models of primitive atmospheres. The photolysis of CO_2 and H_2O will form HCHO, and some may rain out. Pinto *et al.* (1980) have estimated that some 10^{11} moles HCHO per year could be formed even at the present level of CO_2. Linear extrapolation to the estimated level of

Table II. Available Energy on the Primitive Earth.

Source	Energy (mW cm^{-2})	Energy yield (nmol J^{-1})
Far UV < 200 nm	0.004	$1 \times \phi$ [a]
UV 200 - 300 nm	0.4	$2 \times \phi$
Near UV 300 - 400 nm	9	$3 \times \phi$
Visible 400 - 800 nm	70	$6 \times \phi$
Electric discharge	0.0005	10
Radioactivity	0.0001	?
Volcanoes	0.00001	?
Shock waves, meteors, etc.	0.00001	100

Source: Data from Miller and Orgel (1974).

[a] ϕ is the quatum yield of the photoreaction: moles of product/Einsteins of photons absorbed. The energy yield is wavelength dependent ($E = h\nu = hc/\lambda$) for photochemical reactions.

in the primitive atmosphere,1 atm, would yield a fixation rate about a tenth that of modern photosynthesis, 10^{16} moles C per year. However this estimate seems to ignore the photolysis of HCHO itself which would be extremely efficient given its absorption down to 300 nm where the photon flux is 10^3 greater than that required for the photolysis of H_2O and CO_2. Thus the quoted yields should be decreased by this factor.

A critic may argue that more complex molecules will absorb at longer wavelengths, encounter a larger flux of photons and so be destroyed. While this is true for some molecules, many others such as polyenes and heterocyclic aromatic are resistant to radiation. The photoresistance of such molecules can be attributed to two different causes. For loose, floppy molecules, the excitation energy is dissipated as heat in picosecond times. For more rigid, aromatic molecules the excited states live long enough to emit lower energy radiation, fluorescence. These are also the photochemically active molecules. Thus these photostabile molecules would enjoy a form of selection and accumulate. Since they absorb strongly in the UV they would screen the less stablemolecules from this radiation. The photoactive molecules would contribute to the desired chemistry.

In Table III we show the compounds making up the present and a possible primitive atmosphere and ocean together with their light absorptions. The atmospheric components are limited to simple molecules and to absorption in the very far UV. We shall concentrate on the components in the ocean. We see that several components of interest to biology such as sulphate and phosphate ions are protected by the strong absorption by water beyond 185 nm. In fact, the UV absorption by water attenuates rapidly (10 meters) all wavelengths shorter than 300 nm (see below). However the rapid mixing of the top water layer of about 100 m guarantees exposure of the ions to these intermediate wavelengths. The nitrate and ferrous ions will be particularly vulnerable to photolysis and are discussed below. Figure 1 shows the photon flux available to the primitive ocean. The cumulative moles of photons or Einsteins per cm^2 per year are plotted versus the maximum wavelength of cumulation. This is a useful way to approximate the absorbed photon flux by a given molecule. To be exact one should convolute the photon flux with the molecular absorption as a function of wavelength. However since all molecules continue to absorb at shorter wavelengths, give or take a little, and since the photon flux is a rapidly decreasing function with decreasing wavelength, the simple product of the cumulated photon flux and the absorption of the molecule at that wavelength will give a good approximation to the absorbed flux.

The rate of nitrate ion formation from the flux of oxides of nitrogen produced in the primitive atmosphere has been (gu)estimated to be 10^{12} moles per year. The nitrate in the ocean will be photolysed by the UV light. For a source limited photosystem, and including attenuation by the water column, the steady state concentration of the photolyte is:

$$c = s\alpha/\phi\varepsilon I_0$$

where s is the source rate, α is the attenuation of water (10^{-3} cm^{-1} at 350 nm), ϕ is the quantum yield of the photolysis ($\approx$ 0.1), ε is the extinction of nitrate ion (20 M^{-1} cm^{-1}) and Io is the cumulative surface photon flux at this wavelength (Fig. 1) times the cross section of the earth (10^{18} cm^2). The steady state concentration of nitrate ion is calculated to be 10^{-9} M. Assuming the same source rates, the calculated concentrations of nitrate and sulfite ions are similar and are given in the table. The value quoted for ferrous ion is that estimated without photolysis. Using the ferrous ion flux estimated from the Hammersley banded ion formation (also 10^{12}moles per year, see below), its steady state concentration would be $2x10^{-9}$ M. Although the steady state values are small, they represent an enormous flux of photochemically formed reactive intermediates, in fact the total of the ion source rate.

There are two important aspects of photochemistry in solution that we must consider. One is the cage effect and the other is the encounter or diffusion limited rate of reaction.

Reactions of simple molecules in the gas phase often require a third body to carry off the excess energy of reaction liberated by say forming a new chemical bond. The cage effect is caused by the surplus of third bodies in the condensed phase. The energy can dissipate on the femtosecond time scale but the molecular products require almost nanoseconds to change places with solvent molecules, i.e. to escape the solvent cage. Thus some fraction will be lost to recombination. The effect of this factor is contained in the quantum yield of the specified reaction.

For a first order or unimolecular reaction that is all there is to it. But for a second order or bimolecular reaction, there is the rate of their meeting. The fastest rate possible for reaction occurs when the reactants react at their first encounter. For spherically symmetric molecules reacting at the sum of their hydrodynamic radii, the second order rate constant depends only on the temperature and the viscosity of the solvent, $k_2=2kT/3000h$ or $2x10^{10}$ M^{-1} s^{-1} for water at 25°C (Fig. 2). Since an excited state lives at most for a few nanoseconds, efficient reaction requires >0.1 M reactant. Since only unreactive Na^+Cl^- fits this criterion, other paths are required. There are three common possibilities: 1) aggregation or complexes, in which the proximity of the reactants on excitation allows rapid (to femtosecond) reaction; 2) solvent, in which the electron is ejected and trapped in the solvent; and 3) quantum mechanical spin, in which the spin of the electrons unpair say from a singlet state, no unpaired electrons, to the triplet state with two unpaired electrons. The return to the original state can be slowed by a million fold. The resulting millisecond lifetime allows quantitative reaction with substances at the micromolar level. Examples are given in Fig. 2. It is known that the quantum yield of solvated electrons from iodide and bromide anions are respectively, 0.2 (254 nm) and 0.5 (229 nm) at pH 7 (Adamson and Fleischauer, 1975). The common product of reactions of type 2 (See Fig. 2) is the solvated electron, a very powerful reductant. The remaining radical from the photoreactive anion is a powerful oxidant. These need not simply recombine since they are formed at $\approx 10^{-10}$ M. Any organic molecule at a higher concentration would preferentially react with these highly active species. Thus a wide range of redox reactions are possible with these species.

The photochemistry of the modern ocean is completely determined by the high concentration of oxygen, $3x10^{-4}$ M. Reaction with the solvated electrons or triplet states occurs in $(k_2 \times \text{concentration of } O_2)^{-1} \approx 100$ns and produces the strong oxidant superoxide ion or the reactive single state of oxygen. These oxidize the reduced dissolved organic matter producing the prevalent "yellow stuff". The study of this enormously active photosystem has only begun recently. Unfortunately none of this work is directly relevant to the anaerobic photochemistry of the primitive ocean. In addition the oxygen also forms the ozone layer which depletes, or used to deplete, those wavelengths shorter than 300 nm. Thus the modern oceanic photochemistry is attenuated and the synthetic possibilities are minimized. However, in the primitive ocean and atmosphere, these reactions would produce an abundance of molecules useful for the beginning of biogenesis.

One has now arrived at the use of all this photoexcitation: to oxidize and reduce the biochemical progenitors, making them more reactive and storing energy in the products. Examples of this scenario are unfortunately rather rare. Studies of oceanic photochemistry under strictly anaerobic conditions are very limited. Since the ferrous ion was prevalent in the primitive ocean and some work has been done on its photochemistry, we will discuss its reactions.

The photochemical formation of hydrogen from ferrous ion as a function of pH is peculiar, as shown in Fig. 3. The rate decreases from pH 1 to pH 4, then rebounds at pH 6 and more

Table III. Composition of the Present and of a Possible Primitive Atmosphere and Ocean[a]

Atmospheric component	Atmospheric pressure P (atm)		Atmospheric absorption	
	Present	Ancient	Wavelength (nm)	α ($atm^{-1}\ cm^{-1}$)
O_2	0.2	$<10^{-10}$	150	300
H_2	5×10^{-7}	10^{-3}	110	
H_2O	Variable	Variable	170	150
N_2	0.8	1	140	
NO	$10^{-9}(NO_2)$	10^{-9}	225	3
NH_3	10^{-8}	$<10^{-10}$	230	200
CO_2	3×10^{-4}	1	150	15
CO	10^{-7}	$\sim 10^{-2}$	150	2
CH_4	10^{-6}	$<10^{-10}$	130	600
SO_2	10^{-10}	10^{-10}	290	10
H_2S	10^{-10}	10^{-10}	200	150

Oceanic component	Oceanic concentration (M)		Oceanic absorption	
	Present	Ancient	Wavelength (nm)	ε ($M^{-1}\ cm^{-1}$)
Na^+	0.5	0.5	–	–
Mg^{2+}	0.05	0.05	–	–
Cl^-	0.5	0.5	(174)	2×10^4
Br^-	10^{-3}	10^{-3}	198	1×10^4
I^-	10^{-6}	10^{-6}	228	1×10^4
HCO_3^-	2×10^{-3}	~ 1	190	5×10^2
SO_4^{2-}	0.03	10^{-2}	(175)	3×10^2
SO_3^{2-}	~ 0	(10^{-10})	227	$\sim 10^3$
$H_2PO_4^{2-}$	10^{-6}	10^{-6}	(180)	10^3
NO_3^-		10^{-8}	200 300	10^4 10
NO_2^-	4×10^{-5}	10^{-9}	210 355	5×10^3 20
Fe^{3+}	10^{-7}(colloid)	~ 0	320	5×10^3
Fe^{2+}	~ 0	10^{-4}	350	3
pH	8	5-6		

Source: Data from Holland (1984), Walker (1977), and Kasting *et al.* (1989).

[a] The concentration of components in parenthesis are determined in this paper to be photochemically limited. The maxima of the longest wavelength absorption bands and the absorption index α or ε of the components are listed.
The wavelengths in parentheses are absorbed by water, so these ions are protected.

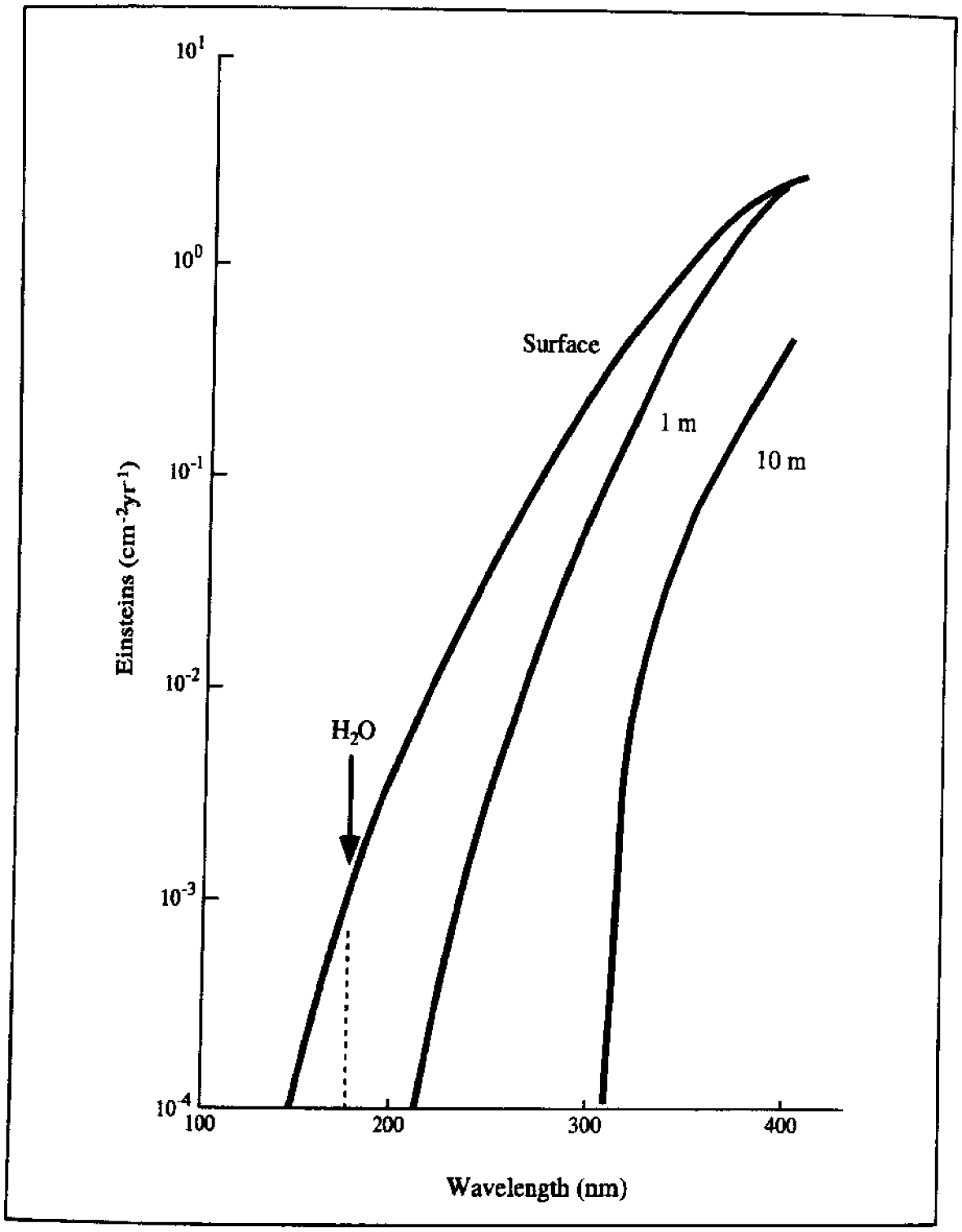

Fig. 1. The cumulated Einsteins (moles of solar photons) available up to a given wavelength plotted on a logarithmic scale versus wavelength at the surface and at 1- and 10-meter depths of the primitive ocean. The cutoff at ~180 nm by a strong water absorption is indicated. The attenuation is based on data of Withrow and Withrow (1956).

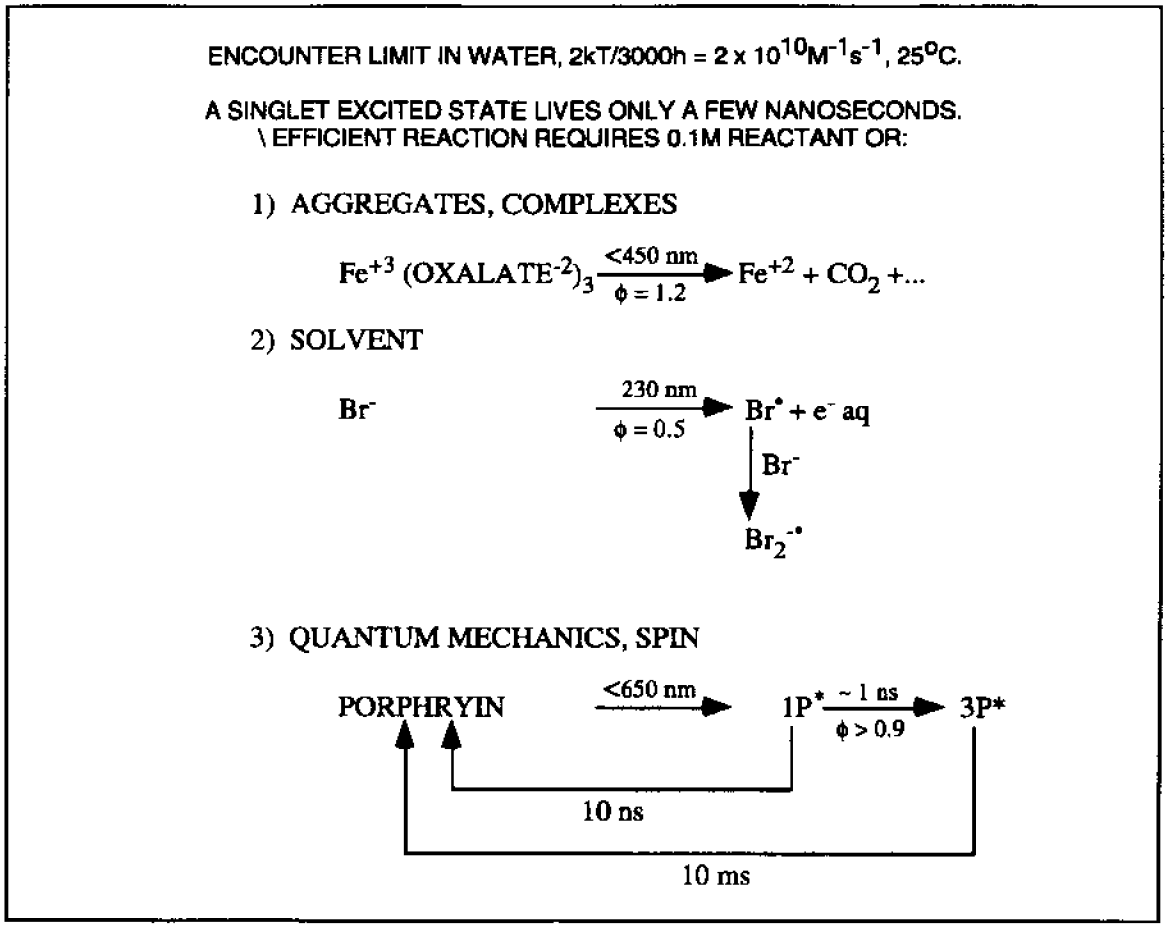

Fig. 2. Photochemistry in solution.

solutions. Replacing the quartz cell with one of pyrex gives the explanation: the reaction in acid requires short wavelength UV, while that beyond pH 6 can make do with wavelengths >300 nm. The reaction is linear in light intensity and the minimum quantum yield, i.e. assuming all light is absorbed, is 0.3 between 300 and 370 nm. The reaction in acid solution from exciting the monmeric hydrated ferrous ion produces hydrogen atoms, which combine to give hydrogen. In contrast, the ferrous hydroxide reaction seems to be concerted occurring from an aggregate to form hydrogen directly (Fig. 4) by reduction of the intermediate by a neighboring ferrous ion. The amount available is appreciable. From the Hamersley basin, well studied by Trendall (1972), one calculates the formation of 3×10^{11} moles of $H_2\ yr^{-1}$. Total emission from volcanoes is estimated to be about the same but the Hamersley basin occupies only 10^{-4} of the earth's surface; many such basins may have been present at one time. The hydrogen would be useful to reduce CO_2. The chemical origin of life needs all the help it can get.

The oxidation of the banded iron formations was originally believed to be caused by the photosynthetically formed oxygen. They would have acted as a vast sink for the gas before it could accumulate in the atmosphere. However the product would then be hematite, as it is in the red banks of age <2Gyr. The photochemical reaction is strongly inhibited by the presence of the ferric ion, and thus cannot go to completion. The banded iron formations have much magnetite, the ferrous-ferric oxide, as expected from this mechanism. At a particular pH, 6.5, ferrous hydroxide can form hydrogen thermally and this reaction also is only partially complete.

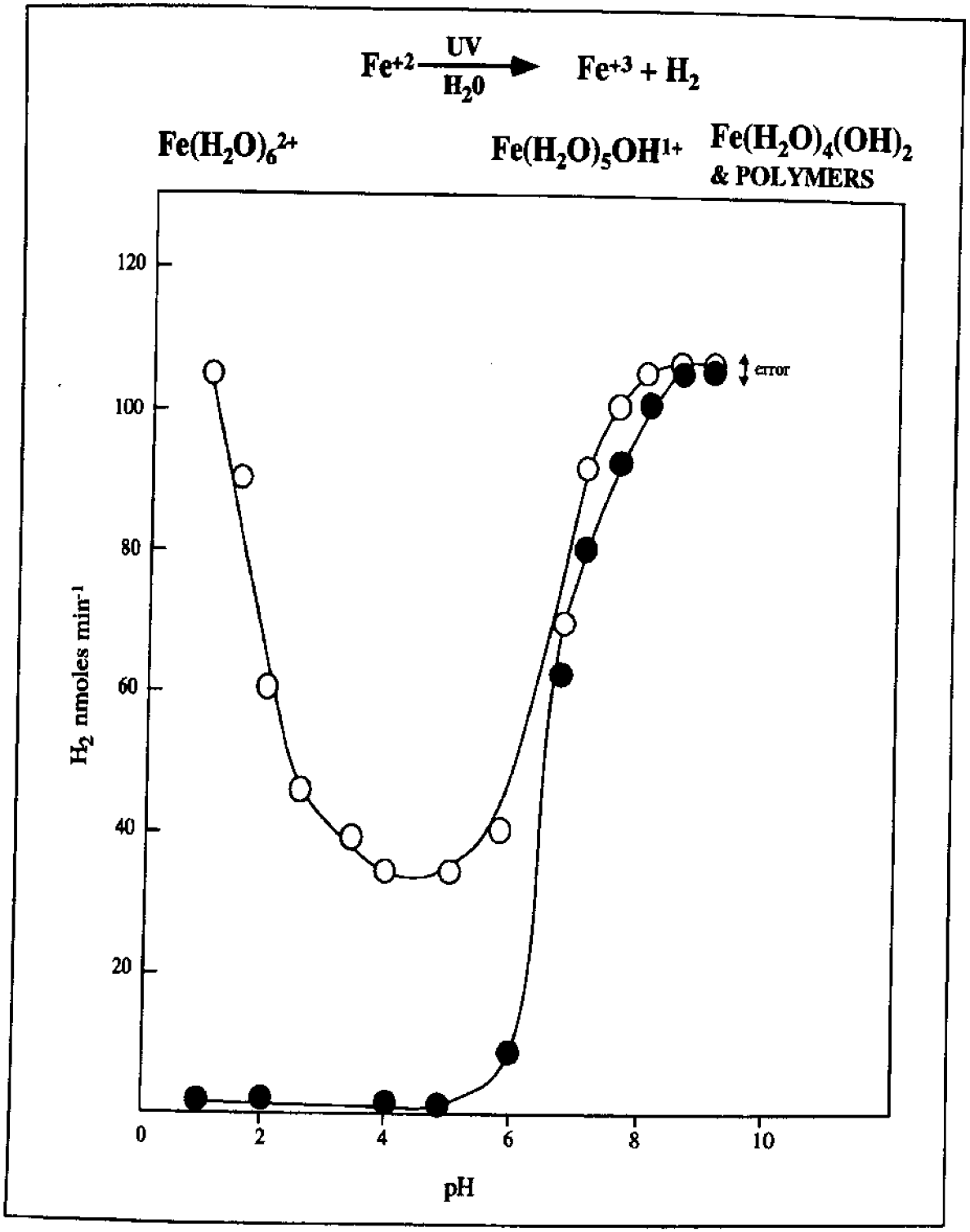

Fig. 3. Photochemical formation of hydrogen from ferrous ion as a function of pH (Mauzerall *et al.*, 1993).

Unfortunately there is no direct reduction of bicarbonate ion by ferrous hydroxide either photochemically or thermally. The reduction is reported to occur in acid solution where free CO_2 alkaline is the reactant and short wavelength UV, 240 nm, is required. There are also reports that ferrous hydroxide can reduce N_2 to ammonia in the dark at pH 8.5. These reactions are critical to the chemical origin of life and deserve further study.

The nitrite anion also absorbs weakly at 350 nm, dissociating to $NO^{\bullet}$ and $OH^{\bullet}$ (on adding a proton). The quantum yield is 0.1, an example of the cage effect. The hydroxyl radical can oxidize all organic molecules including hydrocarbons. Reaction with aromatics results in phenols which are readily further hydroxylated at the ortho or para positions to form o- or p- hydroquinones which are the combined electron-proton carriers in biological systems. The previous estimate of 10^{12} moles per year of NO from atmospheric photochemistry would thus supply a roughly equivalent amount of oxidant to the ocean.

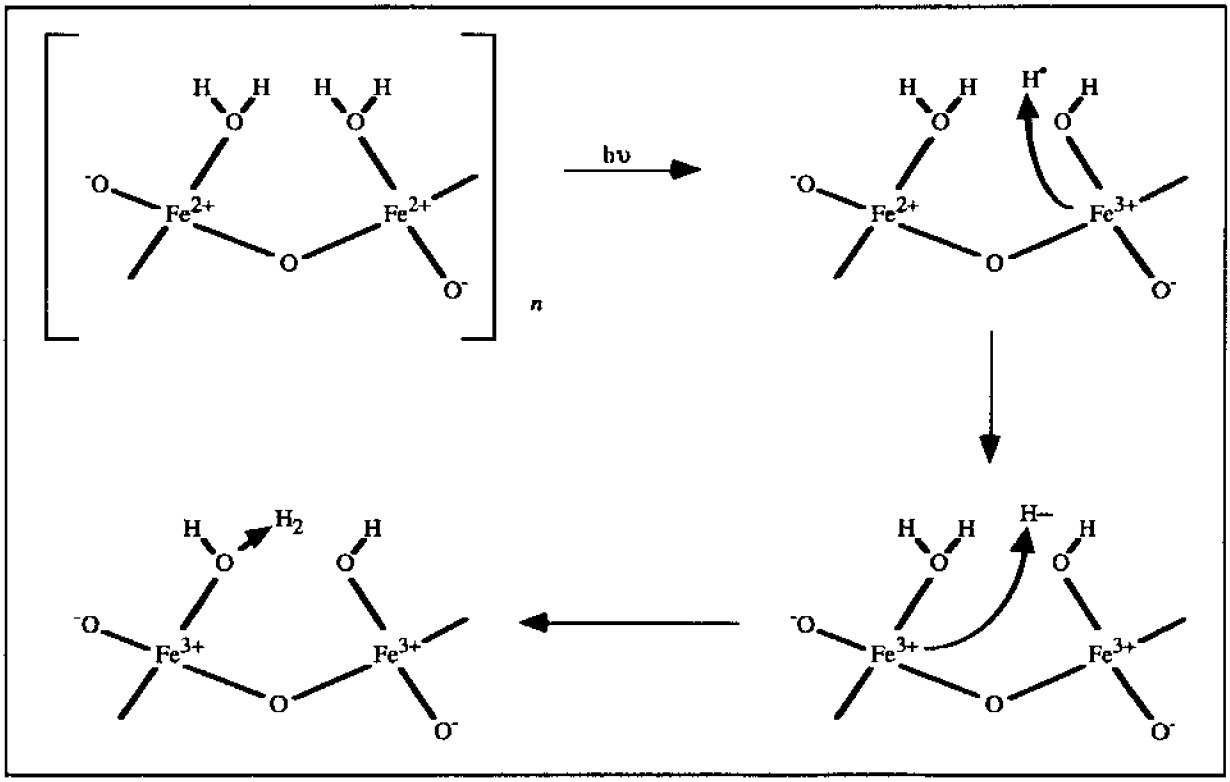

Fig. 4. Direct formation of hydrogen via the ferrous hydroxide reaction. Note the driving force in this reaction: Fe^{+2}-O –> Fe^{+3}-O, ΔH = 15 kcal mol^{-1} (Mauzerall *et al.*, 1993).

CONCLUSION

Photoreactions on the primitive earth would lead to a variety of reactive molecules that would contribute to the chemical origin of life. Whether they would be decisive remains to be seen. A tentative conclusion is that the vast amounts of compounds sufficient to fill the oceans as proposed by the original chemical origin of life scenarios (the "soup") are unlikely to be available without the rapid, early development of photosynthesis.

The next step from the inorganic photochemistry described here would be that based on organic molecules, first absorbing in the UV, then progressing into the visible, culminating in the magnificent porphyrins which opened the flood of all photons accessible in water to the power of evolution. A crucial step was the development of photocycles wherein the excited pigment reacts sequentially with both donor and acceptor thus regenerating the pigment for another cycle of activity (fig. 5). By the addition of antenna pigment molecules the system could make do with a tenth of the maximum available solar photon flux. An even more crucial step was the closing of the feedback loop so that molecules generated by the photoreactions can make themselves form more pigment and other necessary components of the system. Finally (inevitably?) a self-duplicating system capable of selection arose and the rest is history. To paraphrase Lewis Thomas: There may have been elements of luck in the emergence of photosynthetic systems, but once these things were on the scene their evolution was ordained. There was simply no other way to go.

Table IV. Flux and Reservoir of Reducing Equivalents in the Hamersley Basin.

AGE: 2.5×10^9 YEARS

AREA: 150,000 KM^2

TIME OF FORMATION: 10^8 YEARS

DEPTH OF SEA: 200 M

DEPOSITION RATE: 0.5 MMOLE $FE/CM^2/YR$

1×10^{12} MOLE FE/YR

TOTAL FE: 2×10^{18} MOLE FE

2/3 FE^{3+}

THUS AVERAGE CONCENTRATION OF FE: 0.5 MMOLE/20*L*/YR = 2.5×10^{-5}M

PROFESSOR TRENDALL ESTIMATES 0.5×10^{-3}M SINCE ONLY 10% SEDIMENTS

PRESENT TOTAL ATMOSPHERIC CO_2: 6×10^{16} MOLES C

PRESENT TOTAL OCEANIC (HCO_3^-): 3×10^{18} MOLES C

PRESENT TOTAL BIOMASS: 5×10^{16} MOLES C

THUS HAMERSLEY BASIN COULD REDUCE 1/6 OF TOTAL OCEANIC CARBONATE AND TENS OF TIMES THE CO_2 IN THE ATMOSPHERE OR THE PRESENT BIOMASS.

PRESENT PHOTOSYNTHETIC PRODUCTION: 3MMOLE $C/CM^2/YR$ ($4e^-$)

THUS HAMERSLEY PRODUCTION COULD BE 3×10^{-4} OF PRESENT.

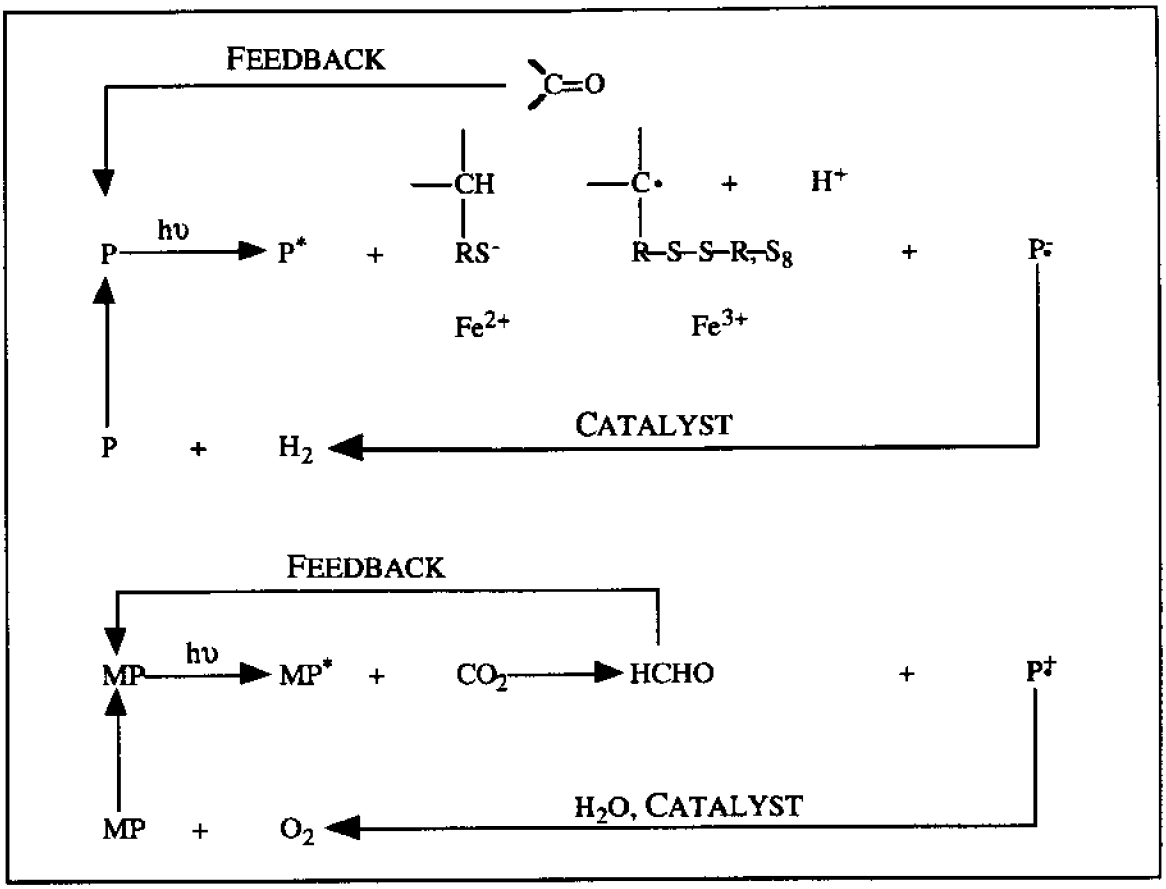

Fig. 5. Evolution of Photosynthesis.

Acknowledgements. Much of this paper was taken from D. Mauzerall, "Ocean sunlight and the origin of life", in THE ENCYCLOPEDIA OF EARTH SYSTEM SCIENCE, VOL. 3, pp 445 - 453, Academic Press, 1992; and from D. Mauzerall, Z. Borowska and I. Zielinski, "Photo and thermal reactions of ferrous hydroxide", Origins of Life, 23: 105 - 114, 1993.

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Oh no, not another workshop: A summary of previous UV workshops

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The following was the opening presentation given to the delegates at the Measurement of Ultraviolet Radiation in Tropical Coastal Ecosystems Workshop held August 3 - 5, 1994 at the East-West Center, University of Hawai'i.

Why was it decided to have a UV photobiology workshop concerned specifically with tropical ecosystems? Recently the focus of UV research has been on polar regions and principally concerned with the Antarctic ozone hole. A 60-70% total ozone depletion occurs during the austral spring. A 5-10% depletion has now been described over the Arctic as well, although that system is a lot more dynamic, and global trends are likely to be about a 2-3% total decrease in ozone heavily weighted at the poles. These small incremental decreases will lead to still higher UV at the Equator. Specifically, while equatorial regions show highly variable decadal decreases in ozone, areas within 20° N show a 12 to 14% decadal decrease in ozone.

INSTRUMENTATION

What kinds of problems do we have with instrumentation, both air measurements and underwater measurements? Do we need full spectral? Do we settle on wide-band radiometers? What kinds of precision are we looking for in our instruments?

Do we create a series of primary centers where we have very sophisticated instruments (as in the network in and around Antartica), and then to a secondary series of places where we are measuring both air and underwater, and measuring perhaps with less sophistication? These measurements could then be complemented with radiative transfer models of the atmosphere and water. How do we inter-calibrate these instruments? This brings up a whole suite of problems.

BIOLOGICAL EFFECTS

What are the biological effects of UV radiation? Most of us here are biologists or photobiologists of one sort or another, so we are very interested in that particular aspect of this workshop and I will comment again on tropical systems as a unique laboratory.

UV radiation is, and has been an important ecological factor. Most of the work in the poles has been stimulated by acute effects, acute exposures to organisms that over evolutionary time have not been exposed to enhanced fluxes of UV radiation. Tropical systems have high biological diversity that has been exposed to high irradiances of UV radiation for a very long evolutionary time. We now have a very unique opportunity to study these systems. The irradiances of UV that occur in tropical ecosystems are much higher than anything that occurs in the Antarctic during the ozone hole. So we have a whole suite of organisms that have evolved various adaptations to protect themselves from the detrimental effects of UV radiation. What sorts of mechanisms have they evolved to protect, repair, and/or avoid ultraviolet radiation?

OTHER WORKSHOPS

What recommendations have come from other workshops? The results of nine previous workshops have already been summarized (Weiler, 1993). It's a very nice document that Sue gave us during another workshop (Lake Lacawac, PA) when we first started to look at freshwater systems If you think there is very little work done on UV photobiology in the tropics or the Antarctic, you should look at the literature on lakes. It is an open field and only a few investigators understand what is going on in lake systems in relation to UV photobiology. There may be some information about UV radiation effects on biogeochemical cycling, but biological

effects on freshwater organisms is completely an largely unknown. Please see the major recommendations that Sue compiled from those workshops.

We should monitor UV and visible radiation using wavelength-specific equipment and establish primary and secondary sites for the monitoring to get long-term data sets. The operative word here is "monitor." The government agencies are also saying we must monitor UV and visible radiation with highly sophisticated instruments but, for whatever reasons, they are not forthcoming with funds to do that. So one of the tasks here, and the task assigned to any workshop (especially the ones from Antarctica) is to provide a convincing document for tropical systems that can be used as evidence that we have a need in tropical ecosystems for this kind of work.

The other major recommendations include obtaining robust action spectra to assess wavelength-specific effects, especially on phytoplankton, the primary producers in the oceans. The action spectra are very important for many reasons, especially for their predictive capabilities. We can also use action spectra for cross-comparisons of laboratory experiments. If we have action spectra we can compare different lamps and different filter combinations that we use in our laboratory experiments because all of us are using different systems.

Using the Antarctic as a biological laboratory for acute effects of experiments of UV radiation, there was a recommendation to try to standardize UV sources, filters, and all other optical equipment that is being used in laboratory-based studies and even extending over into field work as well.

A major concern especially in terms of global elemental cycling, was to look at the study of UV radiation and biogeochemical cycles. A lot of important UV photochemistry is going on in the oceans that may affect the global carbon cycle, for instance. We need to identify cellular targets of UV radiation and study the responsiveness, repair mechanisms, seasonal adjustments, acclimatization response, and evolutionary responses. And the last one that almost all of the workshops talked about was the study of the role of UV absorbing compounds in the system you are working with. In the case of marine systems we have mycosporine-like amino acids (MAAs), but terrestrial systems have flavins and other kinds of compounds that presumably provide some kind of protection. But the evidence here is primarily correlative rather than cause and effect. So there is a need to address that.

Lastly, and again, one of the major points of those workshops was to study ecosystem effects. Recent work by ecologist Max Bothwell (see editorial by Culotta, 1994) points out this need very well, in terms of his studies in freshwater systems. This was a case where he was looking at short-term exposures to periphyton in streams in Canada and found a UV response of decreased biomass and decreased photosynthesis over short periods of time. Serendipitously, the experiment was left running for a longer period of time and what he ended up finding, and publishing in *Science*, was that there was a primary effect of UV on a very important herbivore in the system. And that this effect was more profound than the short-term effects to the algae themselves. It essentially killed the important herbivore, the "keystone species", in that system, and showed that in long-term exposure to UV the algal biomass in those systems actually increased rather than decreased.

So these are very complicated systems. There is a need to take a look at the ecosystem-level and it is very difficult to design experiments because you have to monitor them for a very long time. You have to have the right instrumentation.

We need to provide a good case to funding agencies, to fund photobiology centers like they are trying to do at University of Hawai'i. To fund monitoring networks, not just in the Caribbean basin, but in the Pacific as well. We have to convince our own government agencies that the Pacific is an important theater of study as well as the Caribbean. The Caribbean just happens to be in their political back door.

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General overview of instrumentation, experimental methods, and attenuation of UV radiation in natural waters

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INTRODUCTION

I would like to start off the session with a brief, and general, overview of the field instrumentation that is available and then look at the differences in spectroradiometers versus radiometers. Finally, I would like to present some data that was collected just outside of Kane'ohe Bay that shows the variability in attenuation in various natural water types even here within the tropics.

INSTRUMENTS & HARDWARE

Some of the things we want to talk about in regards to instrumentation is air versus underwater measurements, and the kinds of instruments that are available for those particular kinds of measurements. What should we be investing in, in terms of money, should be the very high resolution instruments for air measurements. We should then be able to model the underwater light field and ground truth that with underwater instruments that may not have quite the resolution, but would be good for monitoring and could be intercalibrated to a good air measurement system and used with appropriate modeling.

The types of instruments that we are talking about fall into two large class groups: radiometers and spectroradiometers. Radiometers are currently represented primarily by the International Light broad band model and the Biospherical Instruments narrow bandmodel. Spectroradiometers, such as LiCor's, have a scanning monochrometer built into it. There are advantages to both of these types of systems. Radiometers in general, like the International Light System, consist of measuring UV radiation within a broad waveband (say for instance, a UV-B instrument that could measure from 290 to 320 nm) and integrating over all of those wavelengths in order to give a single value for that particular wave band. The Biospherical Instrument model has four channels on it plus a PAR channel that integrates over the 400 to 700 nm range. The advantage of some of these instruments is that they are portable and are small. The Biospherical Instrument model gives continuous readings with depth and is very easy to use with a boat. Even though it doesn't give you full spectral information, it does give you information at 305, 320, 340, 380 nm and PAR. The LiCor spectroradiometer is a single monochrometer that gives you full spectral information at the lowest resolution of 1 nm. It works with a monochrometer and a filter wheel assembly that blocks out all of the portions of the spectrum other than the ones being measured at that time. There was some questions about stray light affecting such units but recent comparisons between the LiCor and Biospherical Instruments models show good agreement with each other, and reasonable agreement with radiative transfer models. Sometimes you have to be subjective about the data, especially at the lower end of the spectrum, as you start getting into water that is attenuated. Occasionally you get down to the 300 to 305 nm range of the LiCor instrument and you start getting negative and positive numbers and you know that the data below a certain point is not appropriate so you just have to subjectively select a cut off where you see progressively increasing positive numbers and say that this is good data, this is bad data. This occurs for spectra collected at progressively deeper depths where the shorter wavelengths start to be completely attenuated.

We talked about biological and chemical dosimeters this morning and I put them up here with instrumentation because of some of the factors that we talked about this morning. Ease of deployment, cost effectiveness, and intercalibration needs to be worked out with some of the instruments that we are using in the field. I talked again this morning about the Smithsonian which is in the middle of developing a high resolution 12 channel instrument for measuring ambient levels of solar radiation, UV-B in particular. Recently I was at a remote sensing meeting where a group of people were interested in utilizing remote sensing to do monitoring work on coral reefs. All of the

people involved with remote sensing satellites were telling us what we could and couldn't do, what was real and not real. Dr. Charles Mazel from MIT brought an instrument along with him that I found fascinating for some of the work that we may be interested in as coral reef biologists. It was a hand held spectroradiometer with a hard circuited monochrometer, not a mechanical monochrometer. It was held in two small cases, one was a battery pack and one was all of the instrumentation. It was able to do several things. It was a fiberoptic probe that was hand held and just by a series of different clicks you could get full spectrum information from 275 to 850 nm. I don't know what the resolution of that scan was. With the fiberoptic probe you could get this instrument in hard to reach places. It also had the capability to measure fluorescence as well just by clicking on to another channel. Within seconds you could get both spectra for those of us who are interested in how primary productivity and how fluorescence relates. It was built for a total cost of $10,000 to $15,000. There was a recent instrumentation "shoot-out" at Lake Lacawac in September of 1993 where several people, including the manufacturers, brought representatives of the instruments I just talked about. There were Biospherical Instruments models, LiCor Li-1800 underwater spectroradiometers, biological dosimeters, and an International Light system available. The result of that intercomparison workshop was impressive (Kirk et al. 1994). It talks about the different instruments and their particular characteristics and includes a lot of spectral measurements. You would be suprised, despite the pitfalls associated with any one of these instruments, how close these instruments compared with each other under most situations (especially the Biospherical Instruments and LiCor models).

Again the consensus, in terms of instrumentation, is to have a network of primary monitoring sites with high resolution full spectral capability spectral radiometers very similar to the system established by NSF and Office of Polar Programs (OPP). Those types of instruments are very expensive. Maybe something like the Smithsonian 12 channel could suffice as a high resolution machine for air measurement if it falls within specific guidelines that have been addressed in another workshop on UV-B Radiation. One could take instruments like the broad band radiometer or the LiCor Spectroradiometer, or even the biological or chemical dosimeters, and find a way of intercalibrating and use those instruments as secondary sites for terrestrial and underwater measurements. We need to consider this since we can't afford (and are not going to get the money) to build enough high resolution systems to get the kind of coverage that we want. We will have to use some kind of combination of the three types of systems mentioned above.

EXPERIMENTAL METHODS

In terms of UV photobiology, it is no longer adequate to just describe the lamp system that you are using. The best way to do it is to get a spectrum of that source and publish it along with your experiments. It gives a chance to compare our data with your data. Again action spectra play a role in this as well. The pressure is going to be put on you to provide that information more and more in the future.

We had some good discussion about subtraction and addition experiments and what is appropriate to assess the effects of UV radiation on marine organisms in particular. Most of us do subtraction experiments where we eliminate UV from the spectral regime and look at a relaxation and amelioration of the stress response that we assume is already there. In terms of assessing what the effects of ozone depletion, where we have enhanced fluxes of UV-B, it might be more appropriate (and has been done in terrestrial systems) to develop high resolution systems where we can control incrementally, the increases in UV-B irradiance impinging on the system that we are interested in (instead of removing UV to measure if there is some response). It may be more appropriate to develop a system in which we can impose higher irradiances of UV, even in the tropics, for experimental work.

I think you need an action spectra to obtain biologically effective doses for your system. There is going to be even more pressure for people to develop there own action spectra or to describe their system that they are using with someone else's action spectra for biological effective doses for DNA, erythema, photosynthesis, and then we will be better able to compare those results from laboratory to laboratory or from field situation to field situation.

CONCLUSION

What we need to consider in terms of tropical ecosystems is the following. What kind of monitoring do we want to do? What kind of instrumentation should we consider if we are to establish some type of monitoring network? It doesn't matter if it is in the Caribbean or Pacific theater, people need to consider if it is cost effective, what will work in different nations, what will happen to the data, who will calibrate it, who will maintain it, and what is feasible in terms of getting this kind of data on a long term basis.

EXEMPLARY RESULTS

This is a profile in Kane'ohe Bay (Figure 1) within the Sampan Channel, right in the middle of the bay, within the barrier reef. At the surface on this profile, in terms of UV-B irradiances, we are seeing 2.89 W/m^2 at the surface and at 10 meters we are seeing no UV-B. Someplace between 7 and 10 meters we have tremendous attenuation. We do not see anything below 326 nm at 10 meter in Kane'ohe Bay. Kane'ohe Bay is not pristine tropical waters. It has tremendous imput of sediments, dissolved organics, and high chlorophyll levels, and is better described as a tropical estuary.

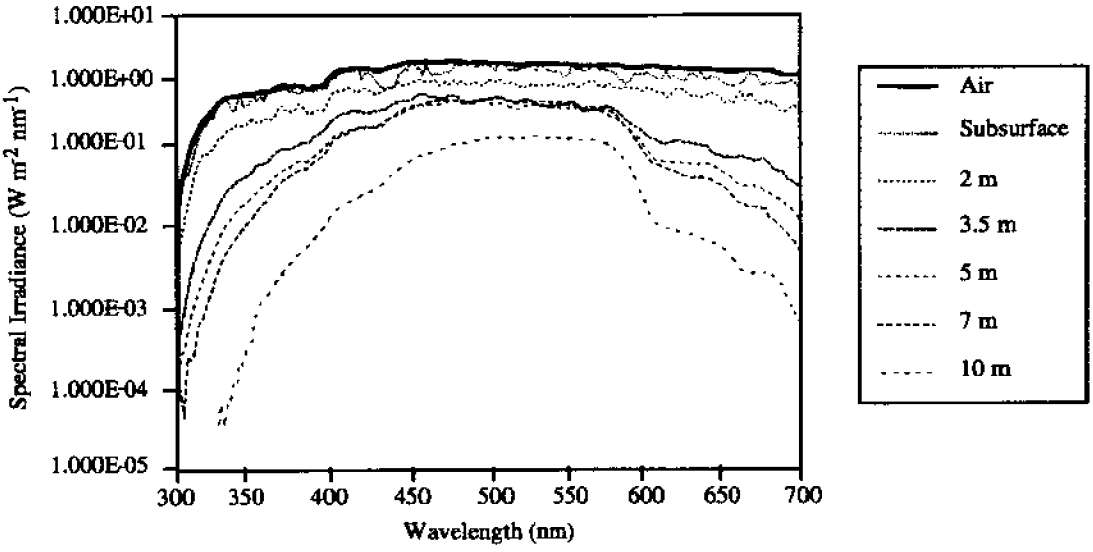

Figure 1. Profile of spectral irradiance (300 - 700 nm) at a series of depths in the Sampan Channel, inside Kane'ohe Bay, O'ahu, Hawai'i.

If we go to a patch reef system north of the previous profile, but still within Kane'ohe Bay (Figure 2), again at 10 meter and below 10 meters we see no UV-B penetration. At this depth we still see a considerable amount of live coral growth. Subsurface irradiance is still at 2.8 - 2.9 W m^{-2} of UV-B. There is some continuity in the attenuation of UV-B and some of this has been verified using a Biospherical Instruments PUV-500 (see K. Patterson this volume) and found that attenuation at 308 is very comparable to what we are seeing using the spectroradiometer, especially the drop between 5 and 10 meters. At 7.5 meters no more UV-B penetrates into these waters.

Waters just outside the bay (Figure 3) are a much different system. Here is found much clearer tropical-like water (case 1 waters), and in this system we are still seeing UV-B penetration down to 18.5 meters (and actually still seeing UV-B irradiances of way less than 0.1 W m^{-2} at 21 meters). Within a very short geographical scale we are seeing dramatic differences in the attenuation of UV-B within and outside the bay. I would like to reiterate the need for not only long term coverage in terms of UV measurements but good spatial coverage in terms of where you are

doing your experiments and what is the question you are asking. I think it is very important to get long term data sets on different spatial scales.

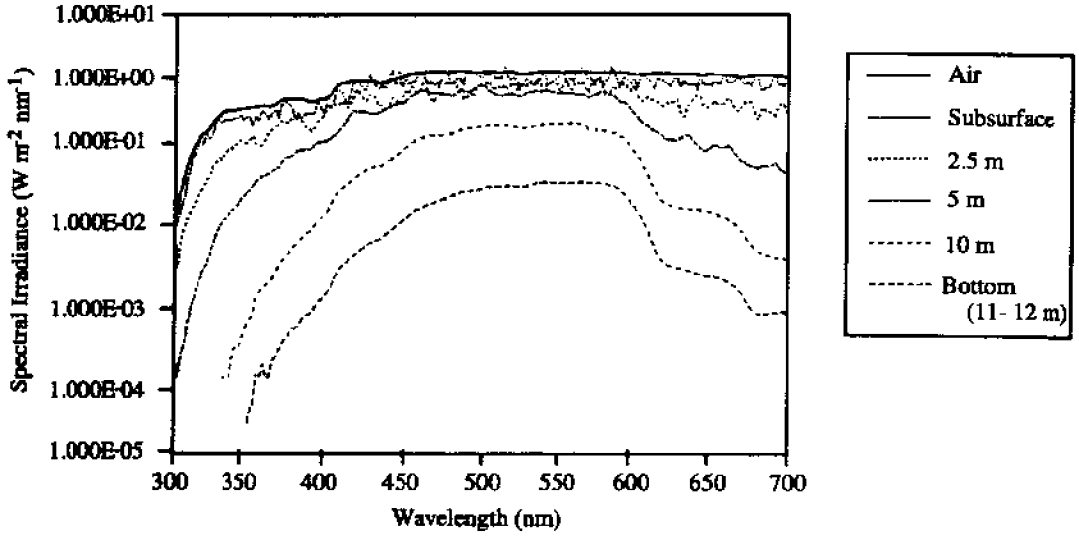

Figure 2. Profile of spectral irradiance (300 - 700 nm) at a series of depths off of the Sliver Reef, inside Kane'ohe Bay, O'ahu, Hawai'i.

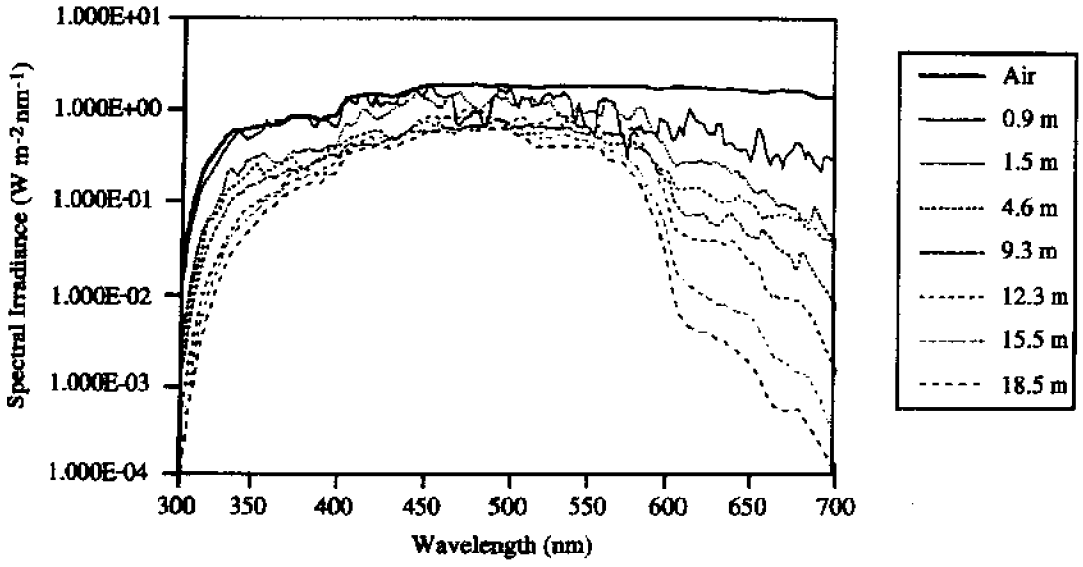

Figure 3. Profile of spectral irradiance (300 - 700 nm) at a series of depths off of Moku Manu island, directly outside of Kane'ohe Bay, O'ahu, Hawai'i.

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Introduction to materials and methods commonly used by participants in the 1994 H. I. M. B. Summer Program on UV Radiation and Coral Reefs

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ABSTRACT: The measurement of the effects of ultraviolet (UV) radiation on tropical marine organisms during the 1994 Hawai'i Institute of Marine Biology's Summer Program on UV Radiation and Coral Reefs involved scientists using a variety of similar equipment and materials to measure incident UV, spectral UV, penetration of UV through the water column and effects of UV on the organisms living in nearshore waters. A comprehensive description is given concerning the equipment used to gather UV information, along with information concerning the plastics used to screen UV and the method used to extract mycosporine-like amino acids (MAAs) from the organisms studied.

INTRODUCTION

The 1994 Edwin W. Pauley Summer Program in Marine Biology held at the Hawai'i Institute of Marine Biology focused on the effects of ultraviolet radiation on coral reefs and associated habitats. Participants in this program used a wide variety of methods to conduct their research. Often the same materials and equipment were used to gather data about incident or spectral ultraviolet (UV) radiation, penetration of UV in the water column or to manipulate UV radiation coming into contact with test organisms. What follows is a basic description of the common equipment and materials used in this research.

UV RADIATION MEASUREMENT:

Data measuring UV radiation was collected concerning three primary areas:

- Incident solar radiation
- Spectral solar radiation
- Underwater penetration of radiation

Incident solar radiation was measured in three different fashions:

1). An Eppley Ultraviolet Radiometer (Eppley Laboratory Inc., Newport, R.I.) was used to measure radiation in the UV-A and UV-B range (280 - 320nm and 320 - 400nm respectively). This machine used a hermetically-sealed selenium barrier-level photoelectric cell whose spectral response was limited to the wavelength interval 295 - 385 nm by an encapsulated narrow bandpass filter. Measurement values were in milliwatts per square centimeter (mW cm^{-2}). This machine had been recently re-calibrated (May, 1994).

2). Both the direct component and the diffuse component of sunlight (that is, irradiance from the sun plus sky), termed Global Solar Radiation, was measured through use of a LiCor LI-200SA Pyranometer (LiCor, Lincoln, Nebraska). Measurement values were in Cal cm^{-2} min^{-1}. This machine had been recently re-calibrated (May, 1994).

3). Photosynthetically Active Radiation (PAR), the energy between 400-700nm that plants use for photosynthesis, was measured using a LiCor LI-190SA Quantum Sensor (LiCor, Lincoln, Nebraska). The sensor measured the Photosynthetic Photon Flux Density (PPFD): the number of photons (between 400 and 700 nm) that came in contact per unit time with a unit area. This machine had also been recently re-calibrated (May, 1994). Measurement values were in µmol photons sec^{-1} m^{-2}.

All of the above measurements were made in air, on a 24 hr basis, from the Point Lab Weather Station located at the Hawai'i Institute of Marine Biology, Coconut Island (Moku o Lo'e), Kane'ohe Bay, Hawai'i.

Underwater and Spectral Measurements:

The majority of measurements were recorded using a LiCor LI-1800UW scanning spectroradiometer (LiCor, Lincoln, Nebraska). This unit measured spectral data (300-700 nm). The cosine-corrected collector and sensors were programmed to scan from 300-700 nm in 2 nm intervals. The instrument was deployed using SCUBA or from a boat at predetermined depths. All measurements of ambient solar irradiance were made at approximately 12 PM. Care was taken to minimize measurements taken during overcast conditions. For each depth threes scans were taken and the mean reported in order to minimize effects of fluctuating radiation. Measurement values were in units of mW m^{-2} nm^{-1}. Integrated values of unweighted UV radiation (300-400 nm) and UV-B radiation (300-320 nm) for each depth were also provided.

PLASTICS:

Plastics used were generally of two types: thin films and thick sheets. Transmission properties were as shown in figs 1-5 (Please note that figs. 1-5 are in % Transmission, not Absorbance; all scans were conducted between 290 and 800nm using a Hewlett Packard 8452A Diode Array Spectrophotometer). Transmission cutoffs for each plastic were defined as the wavelength where 50% of the maximum value was transmitted. Plastics were used to filter solar radiation into the following three categories:

UVT (UV Transparent: UV-B + UV-A + PAR):

- Plexiglas® G UVT Acrylic sheet produced by Rohm & Haas (Philadelphia, PA) 6.0 mm thickness
- Aclar® 33c Fluoropolymer film produced by Allied Signal Plastics (Pottsville, PA) 127 microns thickness (5 gauge)

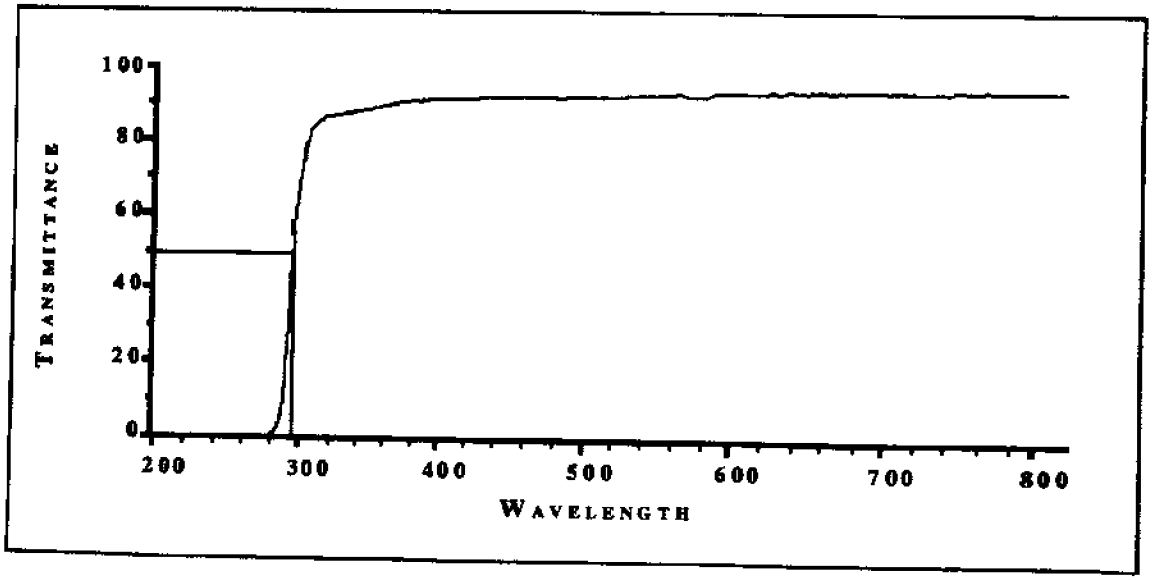

Figure 1. Wavelength scan of Plexiglas® G 6mm Acrylic sheet (UVT sheet); vertical line denotes the 50% transmission cutoff for this plastic.

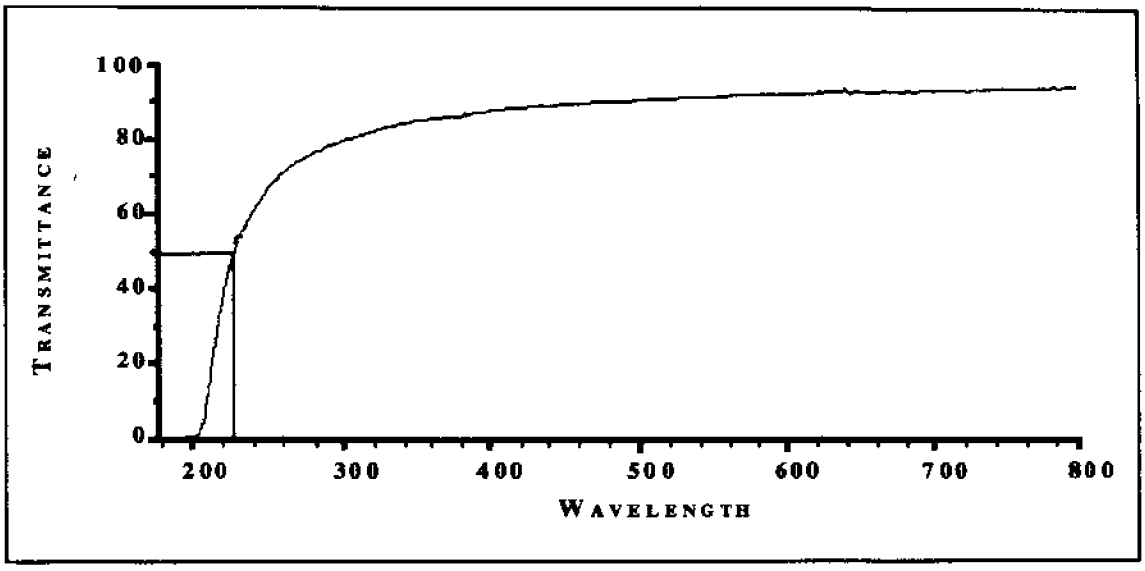

Figure 2. Wavelength scan of Aclar® 33c 5 mil Flouropolymer film (UVT film); vertical line denotes the 50% transmission cutoff for this plastic.

UVA (UV-A + PAR):

- Mylar® Type D Fluoropolymer film produced by DuPont
  127 microns thickness (5 mil)

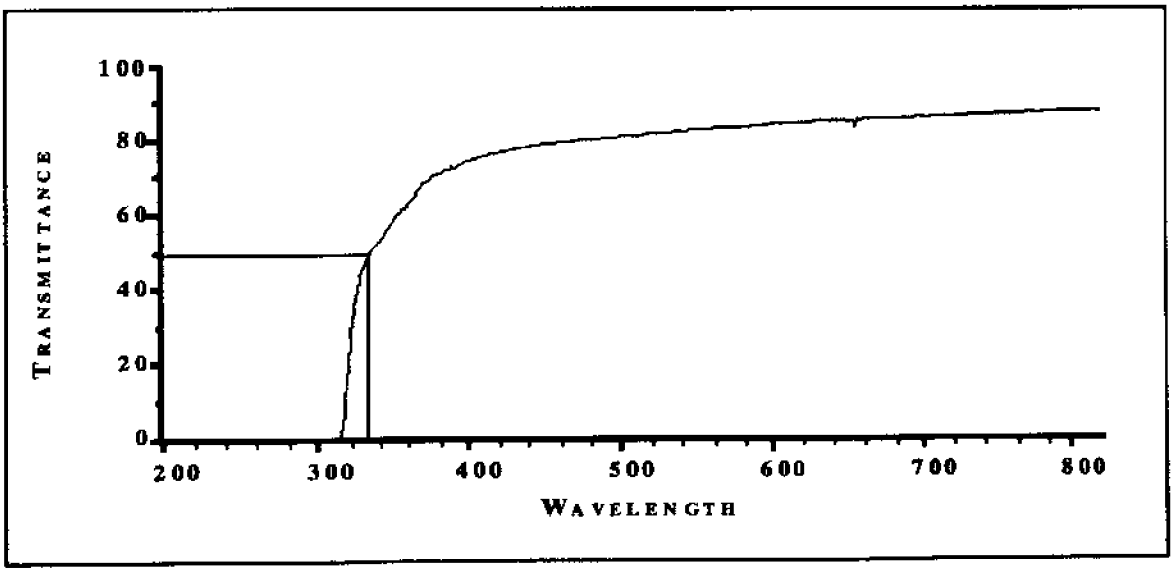

Figure 3. Wavelength scan of Mylar® Type D 5 mil Flouropolymer film (UV-A film); vertical line denotes the 50% transmission cutoff for this plastic.

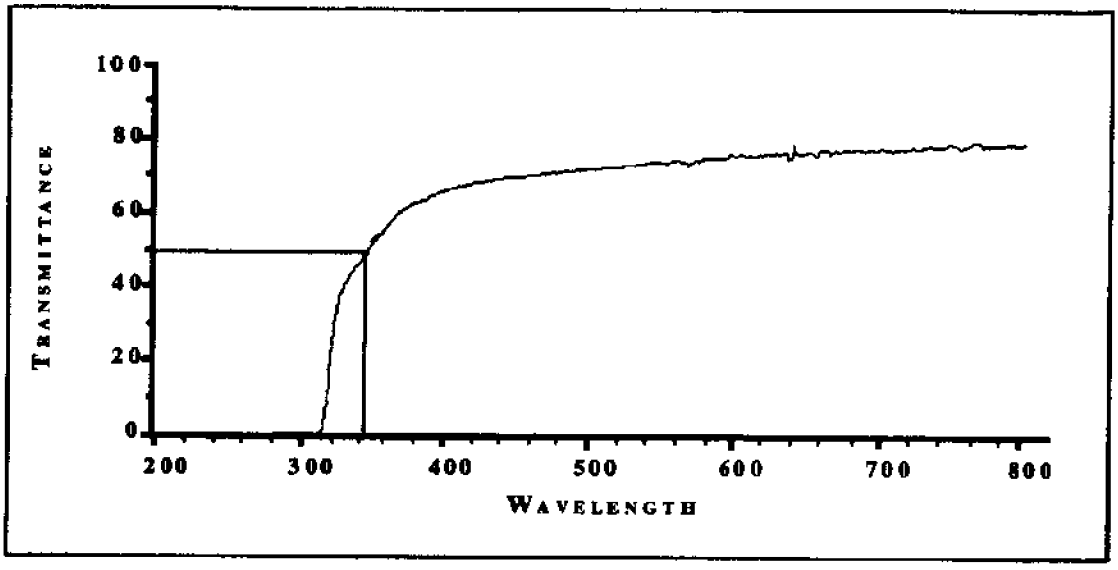

Figure 4. Wavelength scan of Mylar® Type D 5 mil Flouropolymer film (UV-A film) and Plexiglas® G Acrylic sheet (UVT sheet) together; vertical line denotes the 50% transmission cutoff for these plastics.

UVO (UV Opaque: PAR only)

- Plexiglas® G UF-3 Acrylic sheet produced by Polycast Technology Corporation (Stamford, CO) 6.0 mm thickness
- 100% Clear Acrylic Safety Glazing sheet produced by K-S-H, Inc. 2.5 mm thickness

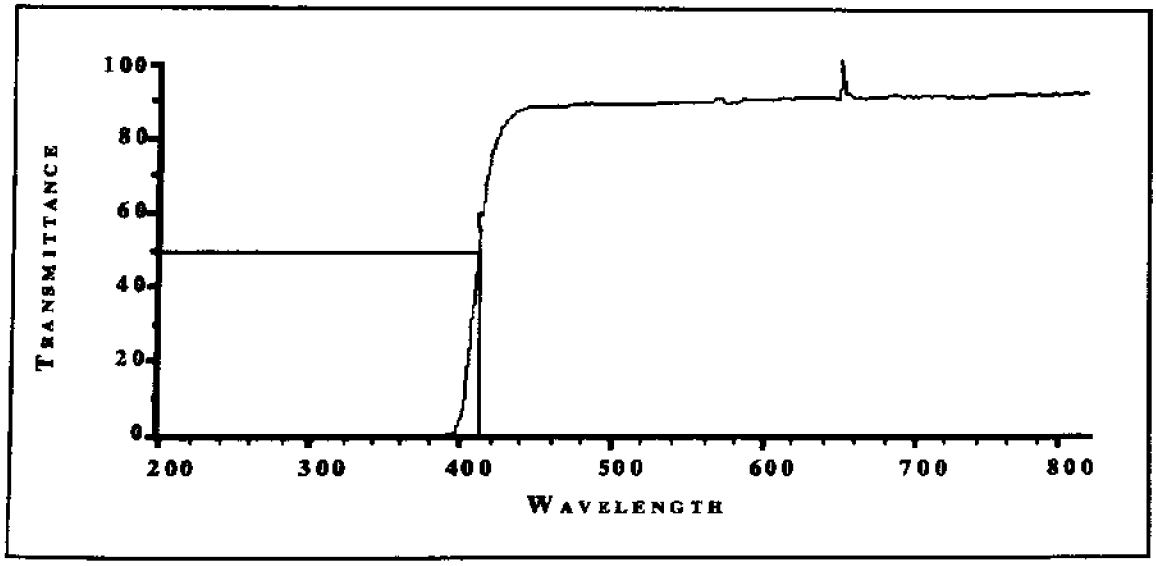

Figure 5. Wavelength scan of Plexiglas® G UF-3 6mm Acrylic sheet (UVO sheet); vertical line denotes the 50% transmission cutoff for this plastic.

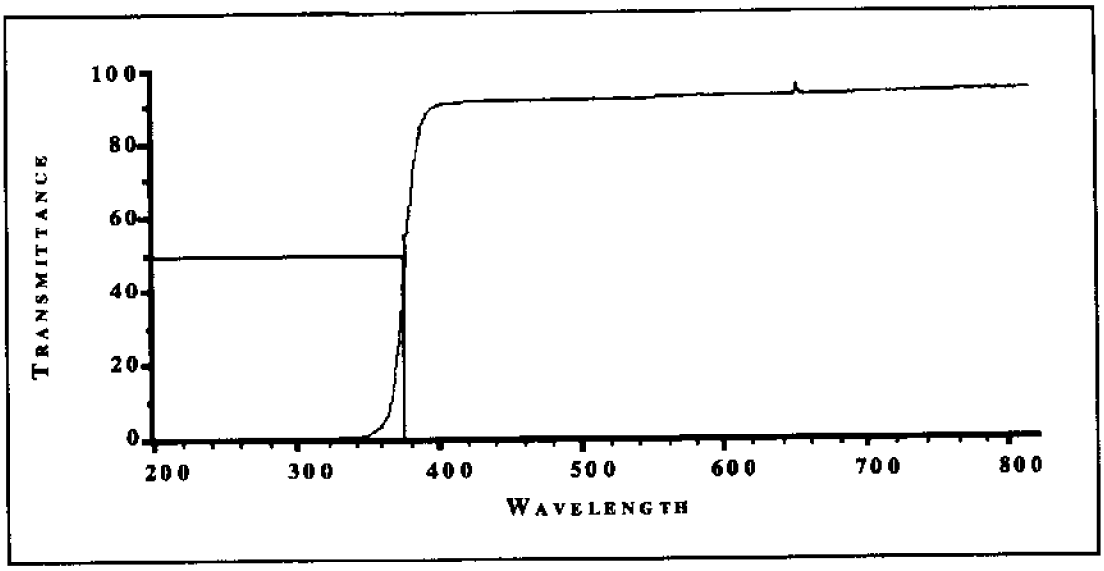

Figure 6. Wavelength scan of 100% Clear Acrylic Safety Glazing 2.5mm sheet (UVO thin sheet); vertical line denotes the 50% transmission cutoff for this plastic.

NEUTRAL DENSITY FILTERS:

Black, plastic mesh of various thickness were used to uniformly decrease the amount of light in order to approximate ambient light levels associated with the depths that the test organisms were gathered from. Scans with the LiCor Spectroradiometer showed that these materials are truly neutral density at PAR and UV wavelengths.

MYCOSPORINE-LIKE AMINO ACIDS (MAAS):

The extraction and analysis of MAAs were performed according to the procedures in Dunlap and Shick et al. 1992 and Stochja et al., 1994. Samples were extracted in 5 cm^3 HPLC grade 100% methanol. Individual MAAs were separated by reverse-phase isocratic HPLC on a Brownlee RP-8 column protected with an RP-8 guard, in an aqueous mobile phase including 0.1% acetic acid and 45% methanol. Detection of peaks was by UV absorbance at 313 and 340 nm. Identities of peaks were confirmed by co-chromatography with standards of mycosporine-glycine, shinorine, porphyra-334, palythine, asterina-330, palythinol, and palythene. Peaks were integrated and quantification of individual MAAs was accomplished using the quantitative standards listed and by on-line diode array spectroscopy. All MAAs were normalized using the soluble protein from an aliquot of the extracted sample. Protein was analyzed using the procedure of Lowry (1951.)

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Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, P. J. (1951). Protein measurement with the folin phenol reagent. *J. Biol. Chem.* 193: 265 - 275.

Stochaj, W. R., Dunlap, W. C. & Schick, J. M. (1994). Two new UV-absorbing mycosporine-like amino acids from the sea anemone *Anthopleura elegantissima* and the effects of zooxanthellae and spectral irradiance on chemical composition and content. *Mar. Biol.* 118: 149 - 156.

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The ultraviolet radiation environment of Kane'ohe Bay, O'ahu

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ABSTRACT: Kane'ohe Bay is a unique calmwater coral reef environment located off the windward side of the island of O'ahu. Data from the Hawai'i Institute of Marine Biology's Point Weather Station were used to look at both annual and daily trends in UV surface irradiance. Factors such as cloud cover, air-bourne particles, and albedo function in modifying the amount of irradiance reaching the surface of the bay. Seasonal variation in UV surface irradiance peaked in June and July and reached minimums during winter months (December through February). Penetration of UV through the water column directly adjacent to the Hawai'i Institute of Marine Biology attenuates quickly and may be impacted to a large extent by both terrestrial and oceanic inputs.

INTRODUCTION

Kane'ohe Bay, on the windward side of the island of O'ahu, consists of a large (31.5 km^2) bay, 12.7 km long and 4.3 km wide (Fig. 1). The landform surrounding the bay is characterized by the steeply-sloped windward side of the Koolau mountain range. The cliff-like mountain faces, termed "the Pali", were initially formed by a massive landslide in which a large portion of the island of O'ahu slipped into the sea a million years ago (Moore *et al.*, 1989), and was subsequently modified by erosion into a series of amphitheater-headed valleys (University of Hawai'i Department of Geography, 1983). The Pali itself provides an abrupt orographic surface for the prevailing northeasterly tradewinds and is an important feature in cloud formation near the bay. The Kane'ohe Bay watershed is made up of seven discrete drainage sub-basins encompassing an area equivalent to 97km^2 (Hunter & Evans, 1993). The ocean-facing side of the bay is bordered by a "barrier reef" with an extensive backreef, while the interior of the bay contains roughly 80 patch reefs of assorted sizes. The internal coastline has a series of fringing reefs, mangroves and ancient fish ponds spread along its length. Two channels provide access into and out of the bay for ship traffic; the ship channel was dredged by the US Navy in 1940 to a depth of 12 m, while the undredged Sampan Channel is only 2 - 3 m deep. Marine water circulation in the bay involves movement of water across the back reef, and then out through either of the two channels; this results in the southern portion of the bay having the most restrictive water circulation with the open ocean. Such a situation makes the southern portion of the bay most vulnerable to the effects of runoff from coastal areas. However the entire bay is extremely estuarine due to restricted exchange with the open ocean.

Prior to the 1950s, the coastal and inland areas fronting Kane'ohe Bay were utilized primarily for agriculture, raising a variety of crops such as taro, rice, sugarcane, and pineapple. Many open areas were used as pastures for horses, cattle and goats (Hunter & Evans, 1993). Distinctive south-north urbanization of the coastline and valleys surrounding Kane'ohe started in the 1960s and continues today. Accompanying this has come a variety of impacts on the bay such as increased sedimentation, freshwater runoff, increased nutrient input, etc. (Banner, 1974; Smith *et al.*, 1981). Sewage discharge directly into the bay from Kane'ohe Town was diverted to a deep ocean outfall in the late 1970s. Sewage from a treatment plant serving the central coastal portion of the bay was diverted in 1986 (Hunter & Evans, 1993). Many coastal areas from the mid- to north bay are still on septic systems & cesspools (P. Jokiel, pers. comm.), and may be contributing to the bay via groundwater.

Annual rainfall in Kane'ohe Bay averages 140 - 240 cm/yr with a daily stream discharge estimated to be around 214,000 m^3 (Jokiel *et al.*, 1993; Hunter & Evans, 1993). Occasional heavy storms result in freshwater floods which inundate the bay. Such catastrophic flooding is rare, occurring once every 20 - 50 years (Jokiel *et al.*, 1993). Still, when floods such as the December 1987 flood occur, they often result in massive die-offs of benthic invertebrates (sponges, corals, zoanthids, etc.) in Kane'ohe Bay (Jokiel *et al.*, 1993; Banner, 1968). Large scale phytoplankton blooms often take place shortly after such flooding events, and may be partially in response to

high nutrient run-off from non-point source outlets such as streams and storm culverts, along with decomposition of dead benthic organisms (Jokiel *et al.*, 1993).

Much of the lagoonal floor is made up of soft mud. Long-term studies on turbidity suggest that waters of the bay, especially the southern portion, are best described as turbid when compared to those directly off-shore (Smith *et al.*, 1981) and is consistent with the effects usually seen with restricted circulation and urbanization influences (Hunter & Evans, 1993). Even so, Kane'ohe Bay is one of the richest coral reef areas in Hawai'i (Jokiel *et al.*, 1994) and is the center for much of the research that is done on coral and coral reefs.

Biologists attempting to understand the importance of UV on the biota of Kane'ohe Bay (and tropical latitudes in general) must have information on spectrum, intensity and modulation of UV input. This paper summarizes available data on seasonal and daily variation in surface incident UV and describes attenuation of UV in the water column of Kane'ohe Bay.

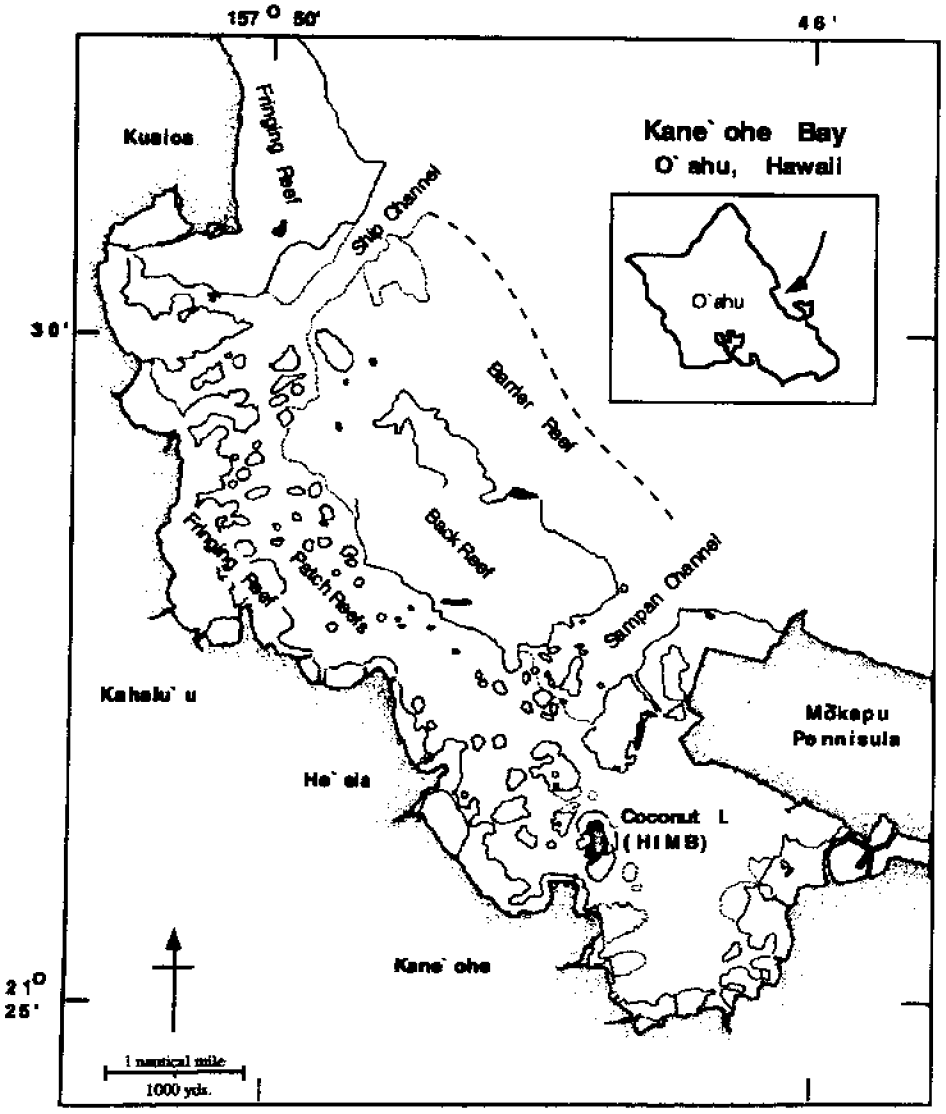


Figure 1. Map of Kane'ohe Bay, O'ahu showing locations of different reef types.

UV PENETRATION THROUGH THE TROPICAL ATMOSPHERE

Solar UV irradiance reaching the surface of the sea is greater in the tropics than in either temperate or sub-polar regions (Calkins & Thordardottir, 1980). Yoshihara & Ekern (1977) calculated that the amount of total solar radiation reaching the surface in Hawai'i was 25% greater than that reaching most areas of the US mainland.

Overall, the solar zenith angle changes with both latitude and time (seasonal and daily); this angle affects the path length that spectral irradiation takes through the atmosphere prior to striking an object and its distribution on the surface. Tropical areas have smaller such angles (at solar noon) than temperate areas (Madronich, 1993), and this contributes to the greater amount of UV present at the tropical sea surface.

Radiation passing through the tropical atmosphere is affected by three major factors: atmospheric gasses, atmospheric particles, and cloud formations (Fig. 2). Atmospheric gasses differentially absorb various wavelengths of spectral irradiance. The gasses of primary importance in this regard are ozone, oxygen, and carbon dioxide. Ozone is the primary absorber of UV-B in the upper atmosphere. More UV passes through the tropical atmosphere because the ozone column is thinner over the tropics than over temperate latitudes (Madronich, 1993). Particles in the air both absorb and reflect radiation, causing a diffusion of the incoming irradiance. The air over most tropical Pacific areas is characterized by very low concentrations of particles (often less than 100) per cubic centimeter (cc), as compared to that found over temperate cities such as Los Angeles or New York (up to 100,000 particles per cc) (Schaefer & Day, 1981) where large densities of urban-derived particles result in the formation of smog. Hawai'i can have a higher particle count than most tropical areas due to a combination of occasional wind conditions and continuous recent volcanic activity producing a natural version of smog called "vog". This vog often causes a noticeable haze throughout the southernmost Hawaiian islands and alters the spectral irradiance reaching the surface.

Particles of air-borne materials can also function as cloud condensation nuclei which result in the formation of clouds (Schaefer & Day, 1981). Cloud formation can occur at various altitudes and densities and are classified into various groups dependent upon their shape and altitude. Clouds often function to decrease the amount of UV reaching the surfaces beneath them. Most of the solar irradiance absorption results from particles other than water in the make-up of the cloud (Madronich, 1993), while most of the scattering of transmitted radiation is due to the water droplets within the cloud. The resulting diffuse radiation that reaches the surface is usually less than 20% of the overall spectral irradiance (Fig. 2).

A final consideration is albedo, the amount of reflected irradiance relative to the amount incident upon a surface. Such reflections, be they from terrestrial, aerial (clouds) or even aquatic sources, can increase the amount of UV radiation both directly and indirectly. Directly, the radiation is increased by increasing the amount of illumination of the target object; indirectly, the radiation can be increased by re-illuminating the atmosphere, which then scatters the reflectance back down towards the surface. Under such conditions, distinctions must be made between localized reflections which primarily luminate only the target area, and regional ones which influence the sky surrounding the target area (Madronich, 1993). Many tropical areas are characterized by oceanic surfaces which serve as strong reflecting bodies; additionally, cloud cover over islands also may serve to reflect measurable amounts of irradiance towards both the atmosphere and towards the surface (other than underneath the cloud). Urban development can result in highly concentrated reflective surfaces (such as concrete, glass, etc.) that can serve to increase the localized albedo within an area.

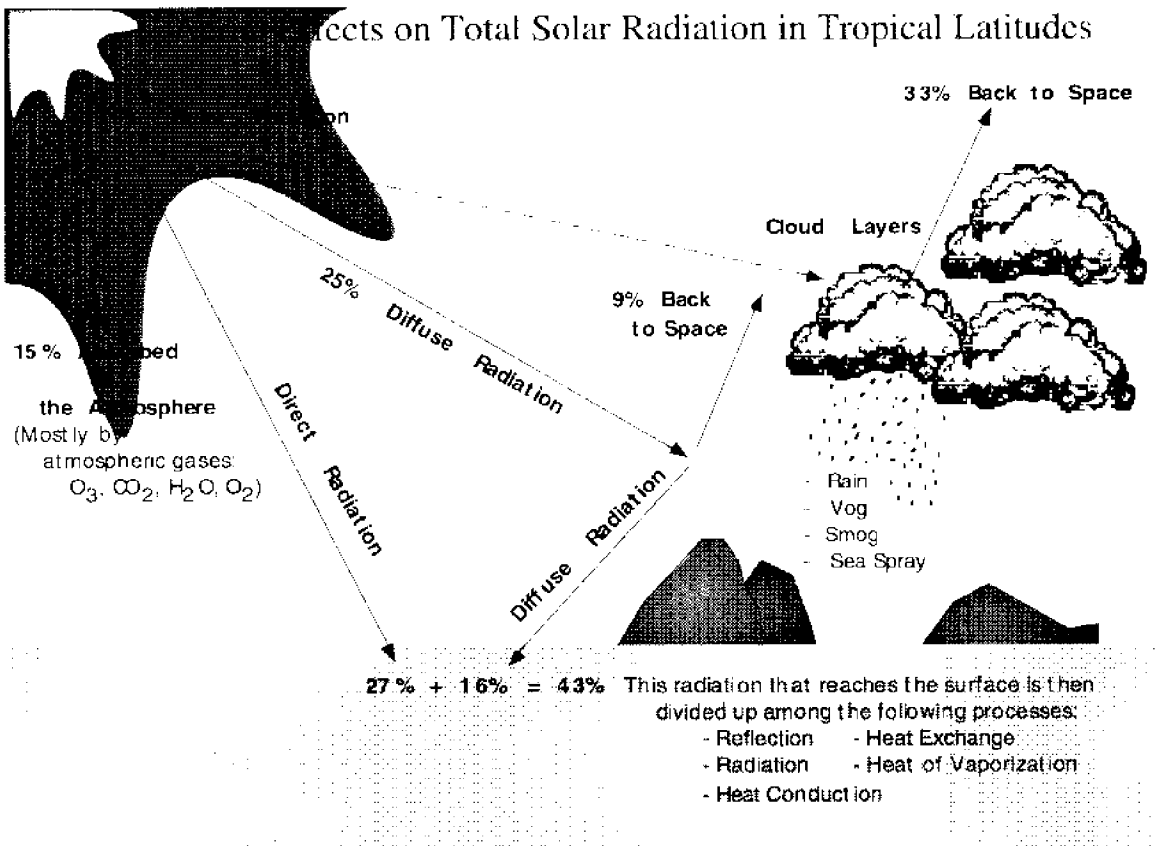


Figure 2. Effects on radiation passage through the tropical atmosphere. Modified from Allen, *Comparative Biochemistry*, vol. 1, edited by Florkin & Mason, Academic Press, New York, 1960, p. 487.

FACTORS AFFECTING SPECTRAL IRRADIANCE REACHING THE SURFACE OF KANE'OHE BAY, O'AHU

The Hawai'i Institute of Marine Biology has had a continuous National Oceanic and Atmospheric Association (NOAA) tide gauge and weather station in operation since the 1950s. Solar data started to be collected in 1970, and since 1985 an Eppley 295 - 385 nm UV radiometer and a LiCor 400 - 700 nm quantameter have been used to collect data which is averaged hourly and stored in a computer database. This database is accessed monthly and printouts provided for use by resident and visiting researchers. Specific information concerning technical features of these instruments is contained within Gulko *et al.* (this volume).

Total radiant exposure, representing daily integration of UV values, is shown for a one year period (1/94 - 12/94) in Fig. 3. Note that the UV sensor was recalibrated during this time period and the data had to be adjusted for this recalibration. The arrangement of the upright sensor precludes detection of UV reflected from surface sources (such as the waters of the bay itself), except where such reflectance is re-reflected by cloud formations back towards the upright sensor. The seasonal trend shown was in agreement with PAR seasonal trends at the HIMB weather station and with previous published radiation measurements for Hawai'i (Yoshihara & Ekern, 1977). Surface UV is highest in the summer months and at a minimum in late December through January.

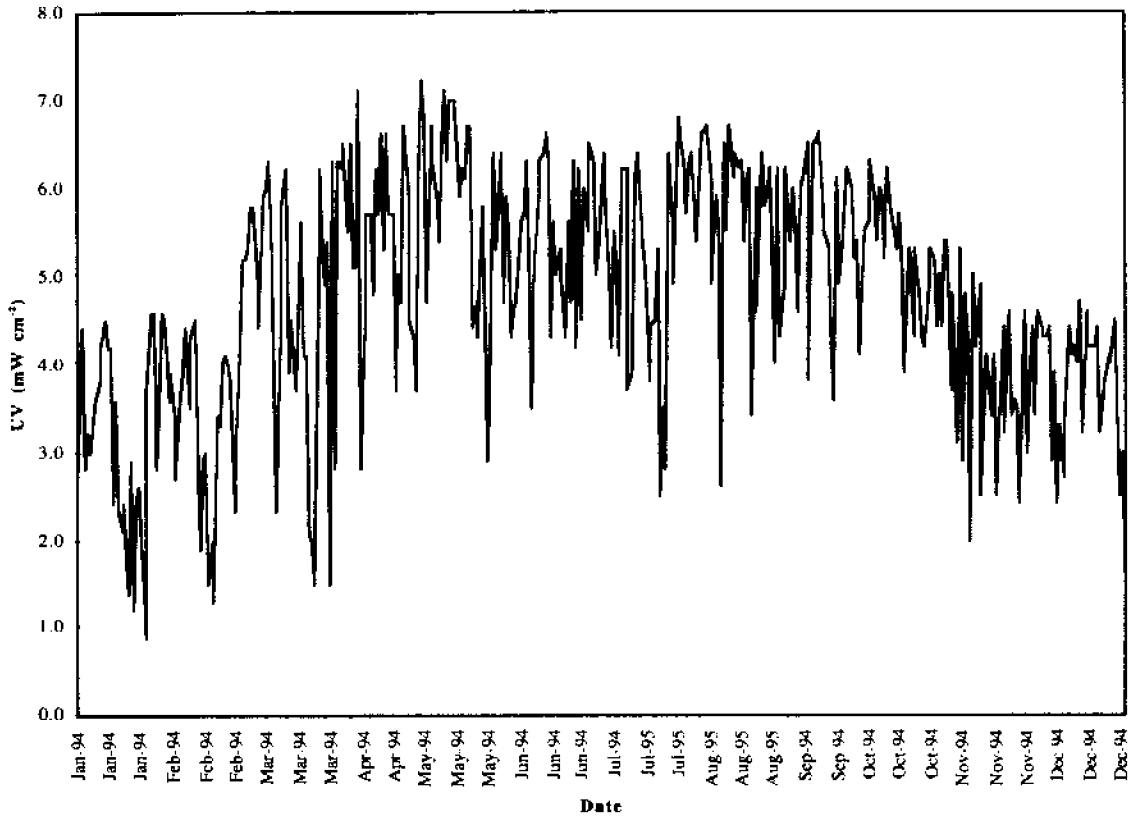


Figure 3. Daily maximum surface UV (mW cm^{-2}) at HIMB, Kane'ohe Bay, Oahu: 1/1/94 - 12/31/94. Data represents mean values taken during hour of highest UV intensity.

Obviously, seasonal shifts in the amount of UV present at the surface will have a direct impact on the amount of UV that penetrates the water column and the biologically effective dose rates that marine organisms are exposed to at any given depth. Many of the experiments described in latter papers in this volume took place between June and August, 1994, in order to take advantage of the high UV levels.

Short-term daily variation (Fig. 3) results primarily from cloud cover and occasionally the relationship between wind condition and the amount of vog present. During periods when the northeast tradewinds aren't active (termed "Kona winds"), strong hazy conditions are present over Kane'ohe Bay (pers. obs.). Other published reports from outside Hawai'i show that at sea level, for the same solar angle (60°), total direct UV irradiance under a very hazy sky (5 km visibility) is roughly one-third of that measured under a clear atmosphere (Kneizys *et al.*, 1988; cited by Mobley, 1994).

Hourly variations in UV levels are shown in Fig. 4 for a randomly chosen week in August 1994. Levels of solar ultraviolet radiation characteristically rise steadily in the morning hours, waver and peak during mid-day, and then decline less steadily in the afternoon. The difference in the fluidity of the afternoon decline may be due in a large part to the common daily formation of clouds over the island of O'ahu.

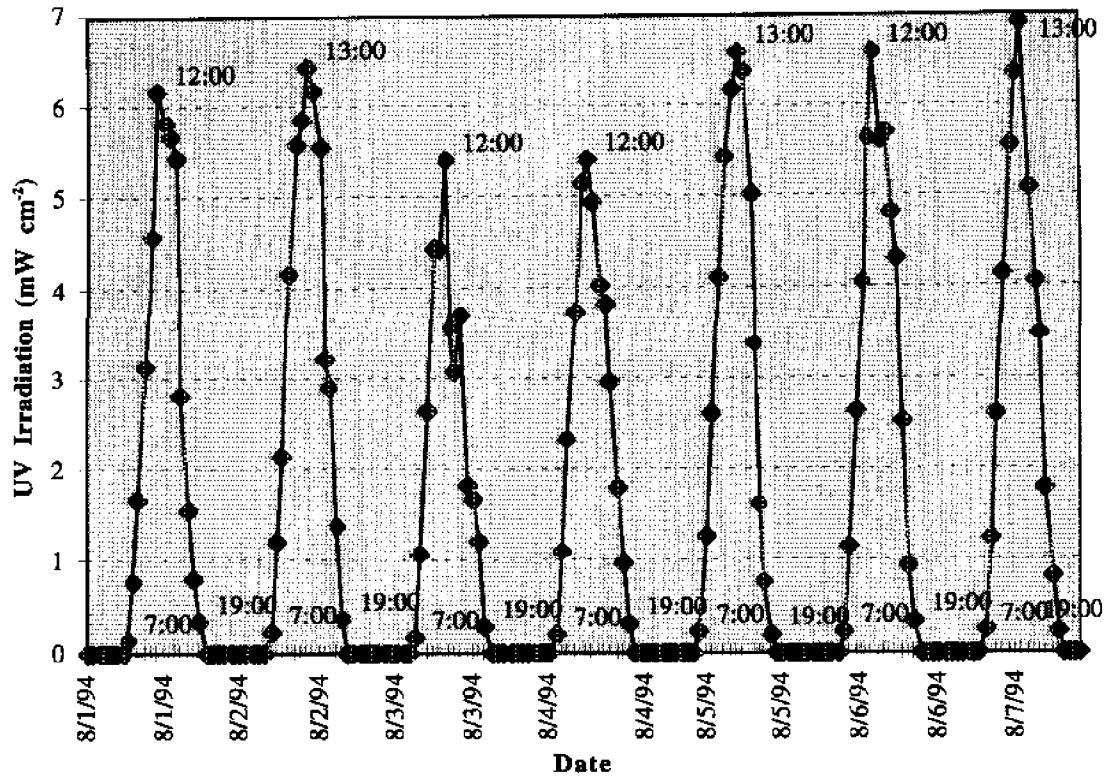
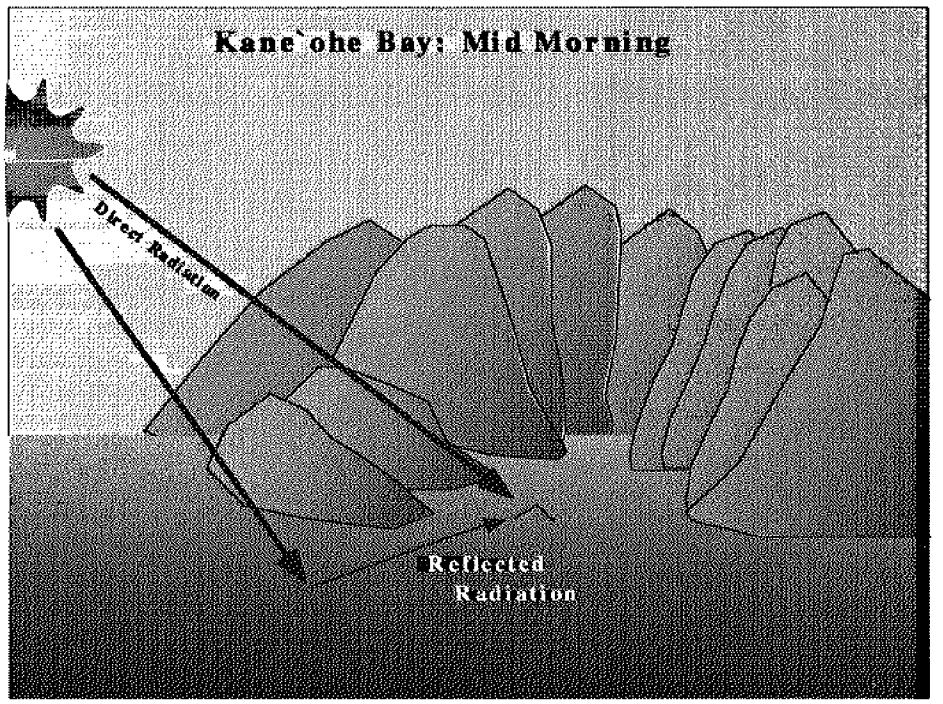


Fig. 4. Mean hourly surface UV (mW cm^{-2}) in Kane'ohe Bay, O'ahu: 8/1/94 - 8/7/94. Data points represent the average value for each hour taken over a one week period. Numbers near each peak represent the time of day for that peak value; numbers near the base of the graph represent the times of day for the first and last measured values.

It is not unusual for daily solar values to fluctuate 25% or more of the mean monthly value (Yoshihara & Ekern, 1977), based on such factors as wind, clouds, etc. Although weather patterns vary, in general, relative humidity on O'ahu is fairly constant at around 80%. The prevailing northeast tradewinds result in a daily pattern of orographic lifting of the moist sea air with associated cloud formation over the mountains fronting the windward side of O'ahu in the late morning and early afternoon. This occurs in addition to seasonal variations in rainfall due to Pacific Ocean influences which result in a characteristic wet season from October to May, and a dry season from June through September (Smith *et al.*, 1981). These daily cloud formations tend to form primarily over the mountains and windward coastal areas and may have the effect of producing an additional atmospheric reflecting body to enhance UV reaching the surface waters of Kane'ohe Bay (Fig. 5). This may help to explain the early afternoon (as opposed to noon) peaks in UV measured on individual days of a given week (Fig. 4).

FACTORS AFFECTING THE PENETRATION OF SPECTRAL IRRADIANCE THROUGH THE WATERS OF KANE'OHE BAY, O'AHU

UV penetrates to considerable depths in clear oceanic waters (Jerlov, 1950). Coastally, the depth of penetration varies with latitude (Smith & Baker, 1979; Calkins & Thordardottir, 1982; Fleischmann, 1989) and season (Levy, 1974). In terms of water properties, UV penetration is dependent to a large degree on turbidity. Turbidity can be influenced by biogenous (i.e. dissolved organic material (DOM), plankton, gelbstoff, etc.) and terrigenous material (Smith & Baker, 1979). Kane'ohe Bay has been characterized as having measurable amounts of terrestrial-



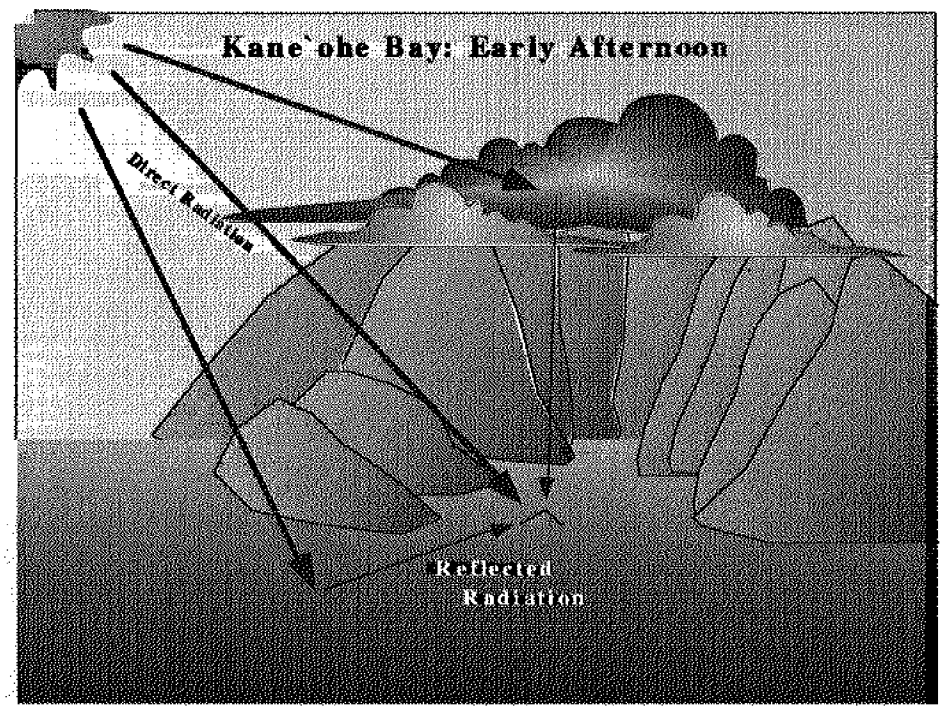


Figure 5. Differences in UV irradiance between mid morning and early afternoon in Kane'ohe Bay, O'ahu.

derived sediment, marine particulate calcium carbonate, organic detritus and plankton. Organic carbon is found at levels four times higher than those directly offshore (Smith *et al.*, 1981).

Penetration of spectral irradiance through the water column was recorded using a LiCor LI-1800UW Scanning Spectroradiometer (see Gulko *et al.*, this volume). Spectral data (300-700 nm) was collected in 2 nm intervals. The instrument was deployed directly off of the Point Laboratory adjacent to the weather station at predetermined depths (above water, directly subsurface, 2 m, 3.5 m, 5 m, 7 m, and 10 m). Ambient solar irradiance measurements were taken continuously around noon at times that minimized overcast conditions. Three scans were taken at each depth and the mean reported in order to minimize effects of fluctuating radiation (Fig. 6).

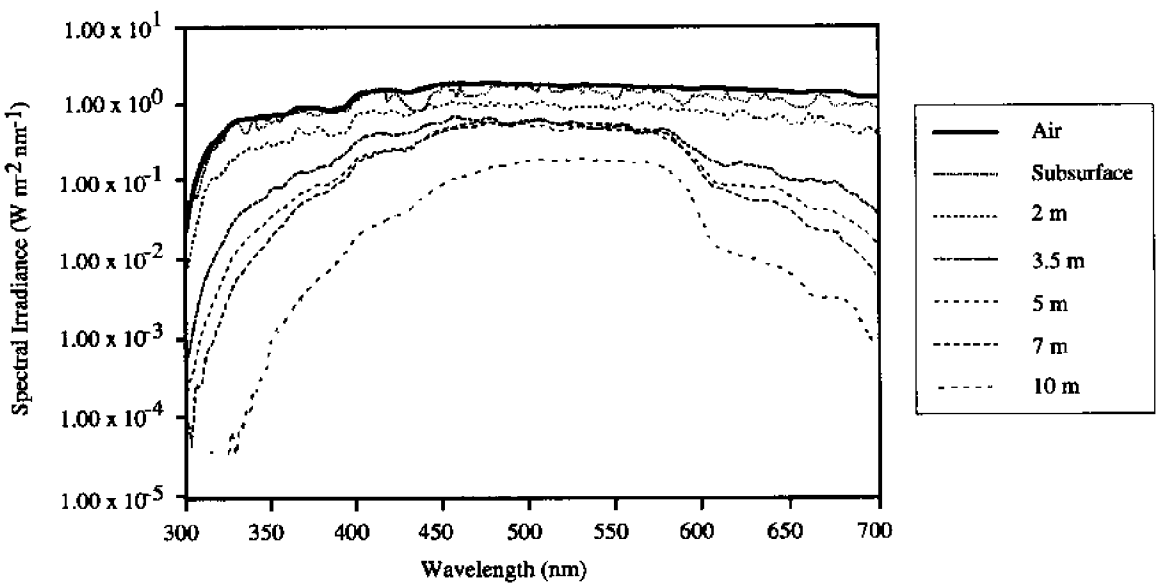


Figure 6. Penetration of spectral irradiance in Kane'ohe Bay; measured around noon on a cloudless July day, 1994.

UV-B penetration in Kane'ohe Bay is relatively limited when contrasted with clear oceanic waters immediately adjacent to it (Lesser, this volume). Yet, the characteristics of spectral irradiance penetration into the water column within Kane'ohe Bay is comparable to, and in-line with, measurements made for other coastal waters (Kullenberg,1982; Smith & Baker, 1979). A more detailed examination of UV attenuation in various sections of Kane'ohe Bay is available in Patterson (this volume).

The diffuse attenuation coefficient for UV (k) is defined as a function of the wavelength of radiation that has the lowest attenuation between 490 nm and 390 nm. This optical property acts to relate subsurface irradiance with irradiance at depth (Smith & Baker, 1979) and decreases exponentially with depth. As UV intensity decreases logarithmically as a function of depth, this relationship can be used to calculate a UV extinction coefficient (K). Larned (this volume) calculated such a coefficient directly adjacent to HIMB within Kane'ohe Bay, to be equivalent to -0.53 · m^{-1}. This suggests that UV attenuates relatively quickly with depth in Kane'ohe Bay. Smith *et al.*, (1981) calculated extinction coefficients for visible light using a secchi disc and a quantum

radiometer; their values for 1979 ranged from -0.25 · m^{-1} for the northern portion of the bay down to -0.41· m^{-1} for portions of the south part of the bay.

Just as solar irradiance transmission through air is affected by particles suspended in the atmosphere, solar irradiance transmission through water is affected by the size, type and distribution of particles suspended in the medium. The most obvious component of sea water, the salts themselves, actually increase scattering uniformly by about 30% over transmission through pure water, and may be involved in increased absorbance of UV wavelengths (Mobley, 1994). At the most basic level, Kane'ohe Bay (which, due to the surrounding watersheds, has varying inputs of freshwater) would be expected to have variations in the penetration of spectral irradiance dependant upon the amount, mixing, and interaction of freshwater inputs. Such an effect would often be overshadowed (pardon the pun) by the amount or types of sediments or nutrients introduced by such inputs.

As stated earlier, a number of studies (Jokiel *et al.*, 1993; Hunter & Evans, 1993; Smith *et al.*, 1981) have shown multiple inputs into the bay which might be expected to affect spectral transmission through the water column. Among these, sedimentation and the effects of nutrients stand out. Dissolved organic matter such as gelbstoff usually originates with decaying plant material (often of terrestrial origin) and absorbs very strongly in the blue and ultraviolet wavelengths (Mobley, 1994). Small, suspended particles would be expected to be non-specific in their reflectance of spectral irradiance overall, in addition to the amount of irradiance that they absorbed. Kane'ohe Bay's waters also support high standing crops of both phytoplankton and zooplankton (Smith *et al.*, 1981); concentrations of plankters would obviously increase attenuation of UV through the water column. Overall, wind speed is thought to play a critical role in determining the amount of resuspended material in the bay's waters (Smith *et al.*, 1981). As wind speed increases, wave action increases, causing dramatic changes in the surface penetration of solar irradiance. High wind speed results in formation of subsurface bubbles which tend to scatter irradiance in all directions (this is why an air bubble produced by a diver appears white underwater) and results in an increase in backscattering of downward spectral irradiance (Mobley, 1994). Such wind-associated vertical mixing would not only effect the depth of spectral irradiance penetration and its intensity, but would also contribute to the types of organisms (primarily planktonic) that were exposed to biologically effective doses. The residence time under which organisms are exposed to higher levels of UV near the surface depends strongly upon the amount of vertical mixing (Kullenberg, 1982). Wind speed also has an effect on wind-generated surface waves within the bay. Such waves contribute to surface scattering of spectral irradiance.

The penetration of UV-B (and to a lesser extent UV-A) has a direct effect on the biologically effective dose rate that marine organisms in Kane'ohe Bay are exposed to and may serve an important role in structuring the distribution of various benthic components within the various habitats found within the Bay.

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Attenuation in Kane'ohe Bay as interpreted from PUV profiles

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ABSTRACT: During the summer of 1994, optical profiles were made in various regions of Kane'ohe Bay. These profiles had a spectral range including the ultraviolet and visible portions of the spectrum. The profiles discussed in this paper were collected with a Biospherical Instruments PUV-500 and are grouped into two transects, one along the major axis of the bay and one along the minor axis of the bay. In general, attenuation increased within a few meters of the bottom of the bay. Profiles collected just outside the bay had markedly lower attenuation than those inside the bay which can in part be attributed to the bathymetric configuration of the area.

INTRODUCTION

To assess the effects of ultraviolet radiation on marine organisms, including corals, and how variations in ultraviolet radiation might effect these organisms in nature, it is necessary to quantify the optical environment in which the organisms live and to know how this environment affects the spectral quality and quantity of radiation received by the organisms. Unlike many other marine organisms, corals are fairly stationary which simplifies quantification of the incident spectral radiation corals receive.

Radiometric measurements assist in the quantification of the radiation environment in which these organisms live. Optical data can be considered in relation to other environmental constituents and variables to obtain insight into the major factors influencing the radiation environment of the organisms and how the radiation environment changes as these factors change. In this paper, I will discuss the general attenuation characteristics of Kane'ohe Bay, Hawai'ias a result of profile measurements made in the ultraviolet and visible portions of the spectrum during the summer of 1994. Results are also discussed in relation to the general bathymetry of the area.

METHODS

During the summer of 1994, an optical survey of Kane'ohe Bay was performed. Included in this survey were profile measurements made with a Biospherical Instruments PUV-500 filter-based radiometer. The PUV-500 makes instantaneous measurements in five spectral bandwidths as well as measuring temperature and pressure (depth). Four of the five spectral channels are ~10nm bandwidths in the ultraviolet portion of the spectrum centered on 305, 320, 340 and 380 nanometers. The fifth channel is a broadband PAR (400-700 nm) channel. Kirk *et al.* (1994) provide a more detailed explanation of the spectral characteristics of the PUV-500 channels. The profiles were made along two main transects which included a range of aquatic environments. Profiles were obtained in areas where the bottom cover was muddy, sandy, and/or coral covered as well as a couple of "blue water" profiles just outside the bay. The approximate locations of the profiles discussed in this paper are shown on Fig. 1. Most of the profiles extend all the way to the bottom. The only exception to this is one profile which was taken just outside the bay and is represented by the northernmost S on Fig. 1.

Raw profile data are not directly comparable due to such things as varying degrees of cloud cover and time of data collection. Wind conditions and sea state were similar over the course of days that data were collected. Because of this, mixing of bottom sediments into the water column by wind-induced mixing was not considered as a concerning factor.

In order to compare the profiles, the following was done. Each profile was normalized to the surface values to eliminate the effects of varying cloud cover over the course of each profile. These normalized values were fitted with an average curve to remove the scatter in the data due to wave action effects. A profile from the southern portion of the bay is shown in Fig. 2 as an example. The average curves were used to calculate attenuation coefficients for each profile at depth intervals of 0.1 meters. New profiles were then created using these attenuation coefficients and a surface "clear-sky" noontime value. This surface "clear-sky" noontime value is the maximum surface value from measurements made on July 30th when the PUV surface unit was placed on the roof of the Point Lab on Coconut Island collecting data every several seconds. This maximum surface value was multiplied by .95 to account for attenuation through the air/water interface. The processed profiles are shown in Figs. 3 and 4. For further discussion, the transect running along the minor axis of the bay will be referred to as transect S and the transect running along the major axis of the bay will be referred to as the transect T.

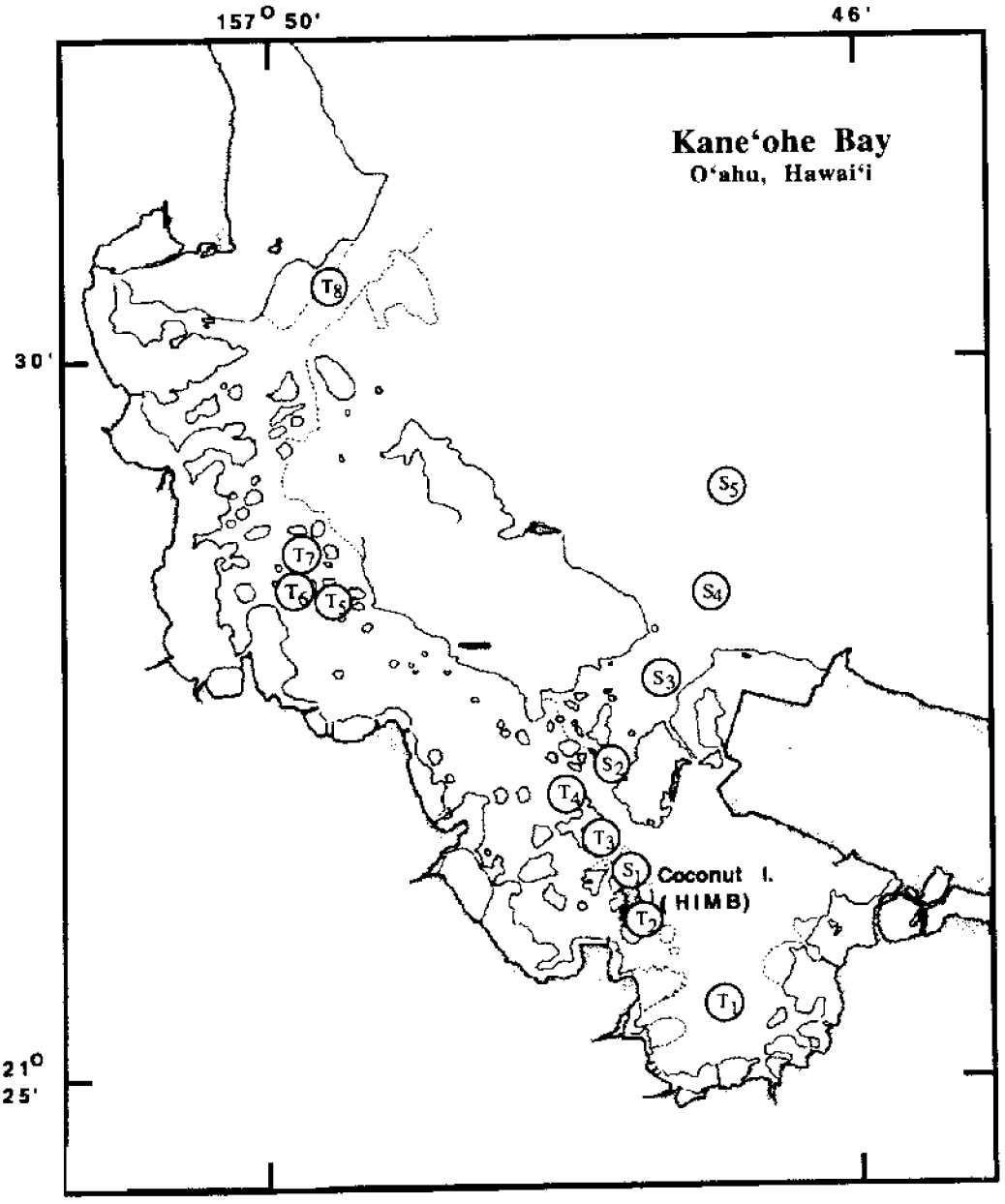

Figure 1. A map of Kane'ohe Bay showing the approximate locations of the profiles discussed in this paper. S's represent the Sampan Channel transect and T's represent the transect along the major axis of the bay.

DISCUSSION

In general, each profile exhibited fairly uniform attenuation down to within a few meters of the bottom. In the few meters near the bottom, attenuation generally increased as shown by an increase in the spacing between meter numbers at depth on Figs. 3 and 4. This is most likely due to an increase in suspended sediments in these deepest few meters. Sufficient data was not collected as to the type of bottom cover to compare the increased attenuation at depth based on the type of bottom cover. However, I would suspect that the profiles with greater increases in attenuation at depth are the ones with a primarily muddy bottom cover rather than a primarily sandy or coral-covered bottom. The greatest attenuation of all collected profiles was found in the second and third profiles of transect S, which were collected in a shallow muddy area.

With the exception of the two transect S profiles just outside the bay (Fig. 4), irradiance at 305 nm fell below the threshhold of detection of the instrument at about 6-9 meters depth, shallower than the depth at which the near-bottom increases in attenuation begin. Irradiance at 320 nm generally fell below detection limits at about 1-3 meters from the bottom and irradiance at 340 nm generally fell below detection limits within a meter of the bottom. With the exception of one profile, irradiance at 380 nm and PAR remained at detectable levels at the bottom.

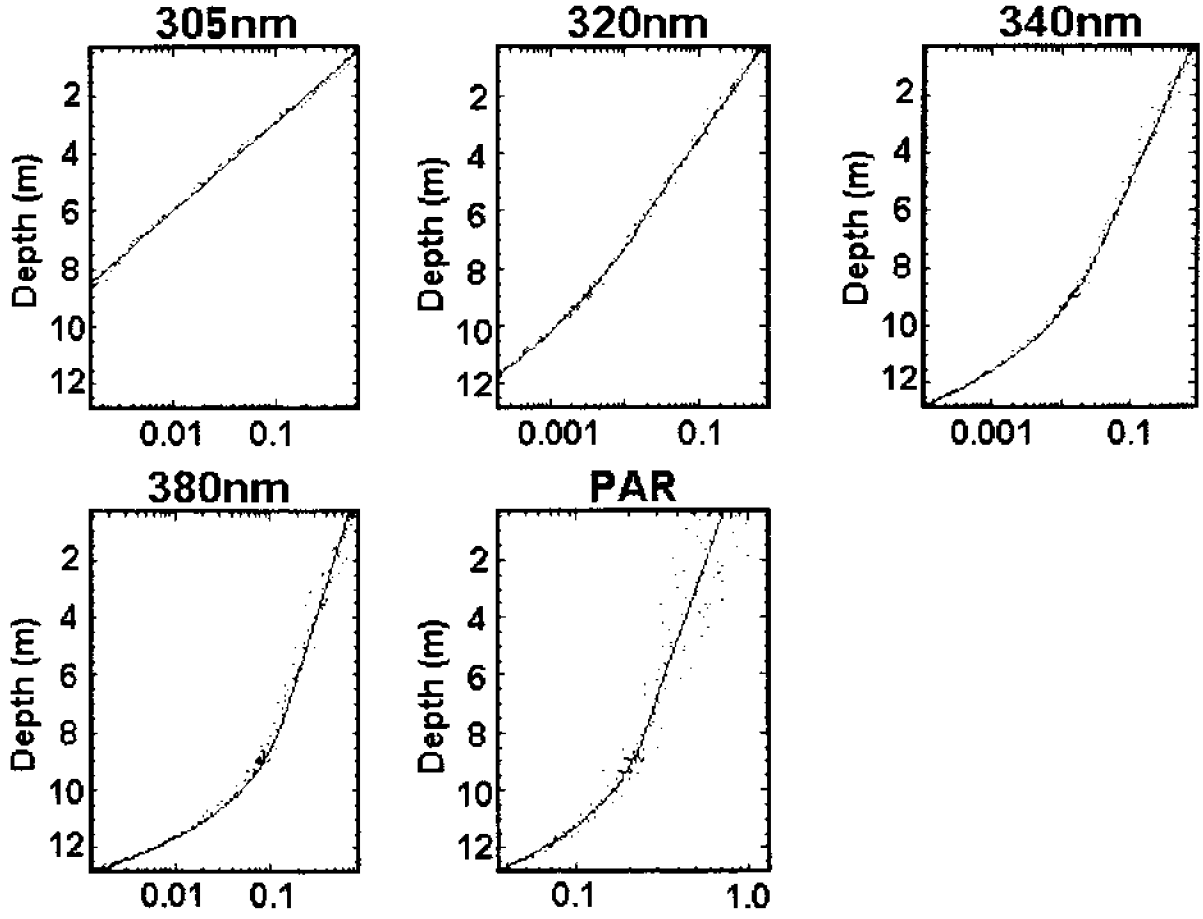

Figure 2. An example normalized curve-fitted profile. This is the southernmost profile in Figure 1, marked by a T. Note the decrease in scatter due to wave action with increasing depth.

At transect T (Fig. 3) there appears to be a trend of increasing attenuation progressing from the more southern profiles to the more northern profiles. The reason for this is not known at this time. The eight profiles represent a range of environments and bottom cover. Without knowing the factors leading to the increase in attenuation in the more northern profiles along this transect, it can not be concluded that this

cross-bay trend is an actual cross-bay trend in the bay and not just the coincidental result of other factors which do not vary in a south to north trend in the bay.

There is significantly higher attenuation inside the bay than outside the bay. The fourth and fifth profiles of transect S (Fig. 4) were collected just outside the bay while all others were collected inside the bay. Irradiances at a depth of 13 meters for these two profiles were, in most cases, at least one to two orders of magnitude higher than the bottom depths of profiles inside the bay (Fig. 4). The waters inside and outside the bay are fairly well separated bathymetrically by a shallow region. The second and third profiles of transect S were collected in this shallow region. The marked difference in attenuation between profiles collected in the bay and those collected just outside the bay can, in part, be explained by this bathymetric barrier with the outer profiles having a much greater deeper water oceanic influence.

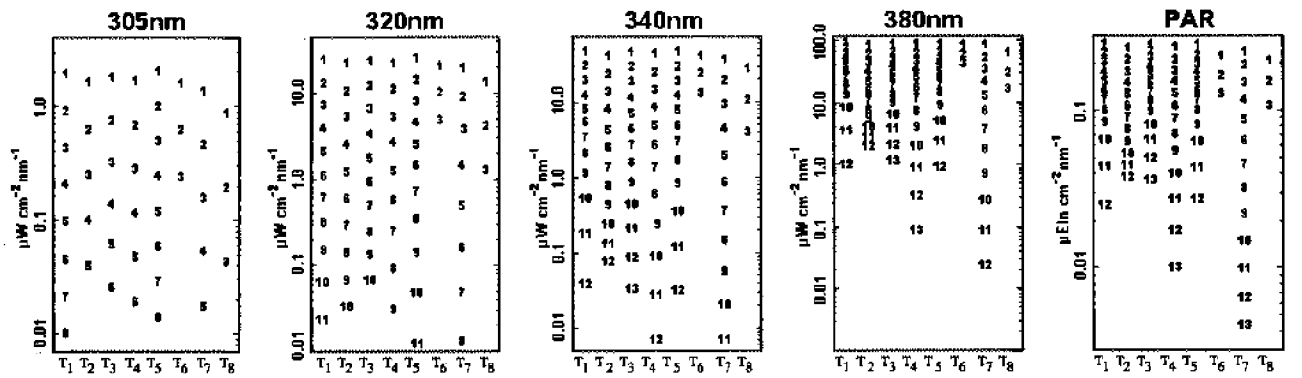

Figure 3. PUV profiles of the T transect at the four UV wavelengths and PAR. The approximate locations for the profiles are shown in Fig. 1. Each column (T_1 through T_8) represents a separate profile, in order, from south to north. The amount of radiation is shown on the y-axis. The numbers plotted represent depth in meters. Note T_8 and T_6 were taken at locations where depth was less than 4 m.

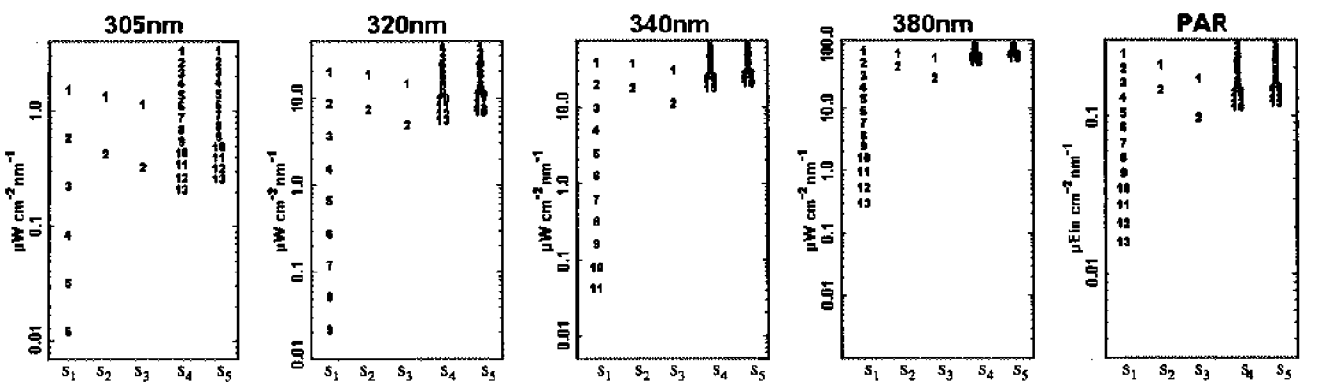

Figure 4. PUV profiles for the S transect at the four UV wavelengths and PAR. The approximate locations for the profiles are shown in Fig. 1. Each column (S_1 through S_5) represents a separate profile, in order, from inside the bay to just outside. The amount of radiation is shown on the y-axis. The numbers plotted represent depth in meters. Note S_2 and S_3 were taken in the Sampan Channel where depth was less than 4 m.

The scatter in the data due to wave action was removed to aid in the comparison of profiles; however, it should not be neglected. The scatter due to wave action decreases with increasing depth (Fig. 2). The scatter in the PAR (400-700 nm) figure is greater mainly due to a wider spectral bandwidth. The other figures represent a 10 nm bandwidth rather than a 300 nm bandwidth. This decrease in wave action effects with increasing depth may be an important factor to consider when analyzing the effects of ultraviolet radiation on corals in some experimental setups. Tanks are generally shallower than the depths at which coral specimens are collected. Tanks also generally have water flowing through them which can create ripples across the surface of the tank similar to small ripples on the sea surface. Also, some experiments have been done where corals are transplanted from a deeper to a shallower depth for the

purpose of assessing the effects of increased ultraviolet radiation on corals(Gleason & Wellington, 1993; Gleason, 1993). When corals are moved from deeper to shallower depths, spectral quantity and quality change. This can be compensated for by using a combination of filters. Wave action scatter is greater at shallower depths and the magnitude of variation in very short-term radiation doses increases. Such wave scatter radiation is generally not compensated for, nor generally addressed as a concerning factor, in the interpretation of experimental results.

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The Influence of Solar UV-B Radiation on Copepods in the Lagoon at Coconut Island, Hawai'i

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ABSTRACT: The influence of solar ultraviolet radiation on copepods was determined in the lagoon at Coconut Island, Hawai'i. The influence of UV-B on egg hatching rate was determined using *Labidocera madurae*. The eggs were incubated in quartz bottles under natural solar radiation. The dose of UV-B was controlled for the duration of the investigation by covering the bottles with a lumilar sheet which absorbed solar radiation shorter than 315 nm. The influence of UV-B on survival rate was determined using nauplii of *Oithona* spp. Survival rate of the nauplii did not decrease with UV-B radiation. Nauplii in the experimental bottle with UV-B radiation molted to copepodid stages at a rate similiar to the those in the control bottle without UV-B. These results indicate that copepods distributed in the lagoon at Coconut Island are well-protected from present-day levels of UV-B radiation.

INTRODUCTION

Copepods are the most dominant zooplankton and play an important role in marine ecosystems. It is known that UV-B radiation influences their survival rate (Dey *et al.*, 1988), fecundity and egg hatching rate (Karanas *et al.*, 1981). In oceanic waters, many copepods may avoid UV radiation through diel vertical migration behavior. They distribute throughout the deep layers during the day and migrate to the food-abundant surface layer during the night. Copepods can not, however, avoid UV radiation by vertical migration while in shallow environments such as lagoons, estuaries, or tidal ponds. It would, therefore, be expected that copepod distribution in shallow environments would be either severely influenced by solar UV-B radiation or they would be well-adapted to the photo-environment and little influenced by present-day levels of UV-B.

In this study we determined the influence of UV-B radiation on copepods using egg hatching rate and nauplii survival rate in the shallow lagoon at Coconut Island, Hawai'i. Animals in their early life stages are usually more vulnerable than their later stages (Damkaer *et al.*, 1980). The results obtained, therefore, were expected to be indicative of the most sensitive effects of UV-B radiation relating to copepod distribution in the lagoon at Coconut Island.

MATERIALS AND METHODS

Experiments were carried out in the lagoon at Coconut Island, Hawai'i (N 22^{O} 26', W 157^{O} 47'). During the experiments underwater photosynthetically active radiation (PAR) and UV-B radiation were measured continously with a Biospherical Instruments underwater spectroradiometer PUV-500. The PUV-500 measured UV-B radiation at 305 nm, 320 nm, 340 nm, and 380 nm spectra. PAR and temperature were also measured. The sensor was set at a depth of 15 cm in the lagoon.

Egg hatching rate of *Labidocera madurae*

Labidocera madurae, the dominant copepod species in the lagoon, was used to investigate the influence of solar ultraviolet radiation on the egg hatching rate. A pump was used to collect zooplankton during the night of July 28, 1994 and *L. madurae* females were sorted out under the microscope. The females were individually placed into 30 ml vials filled with filtered (0.2 μm Nucleopore filter) seawater and maintained for 8 hours. Before sunrise on July 29, the produced eggs were sorted and three groups of 50 eggs were each put into individual 250 ml quartz bottles filled with filtered (0.2 μm Nucleopore filter) sea water, placed just before sunrise into a water table containing flow-through seawater, and maintained for 48 hours. The hatching time of *L. madurae* was between 30 and 36 hours at ambient temperature. UV-B dose was controlled for the duration of the investigation by covering the bottles with a lumilar sheet (Table I) which absorbed wavelengths shorter than 315 nm. As a control, a dark bottle was prepared and all three bottles were covered by the lumiliar sheet throughout the experiment. Nauplii and unhatched eggs were counted after incubation.

Table I UV-B dose to the eggs of *Labidocera madurae*.

Exp.bottle	Duration of UV-B exposure		Dose (mW $cm^{-2}nm^{-1}$)	
	Start	End	305nm	320nm
Control-light	-	-	0.0	0.0
Control-dark	-	-	0.0	0.0
E1	14:00	19:30	15.3	32.5
E2	13:00	19:30	19.8	42.1
E3	12:00	19:30	26.4	56.2
E4	11:00	19:30	34.5	73.4
E5	10:00	19:30	40.8	86.8
E6	5:55	19:30	47.0	100.0

Survival rate of *Oithona* spp. nauplii

Surface water was collected with a bucket and filtered with 100 µm nylon mesh to exclude the larger zooplankton. Filtered sea water was placed into quartz bottles and kept under natural solar radiation in water tables with flow-through sea water. Six experimental bottles and three dark control bottles were prepared and covered with a lumiliar sheet. The experiment was started at 6 am on July 27, 1994. Two experimental bottles and one control bottle were recovered every 24 hours for three days. Water in the bottles was concentrated to 20 ml with a 20 µm nylon mesh and put into 20 ml glass tubes. A lamp was placed at the top of each tube to help distinguish living-active from living-inactive and dead animals. Living-active animals concentrated toward the lamp by their phototaxic behavior while living -inactive or dead animals sunk to the bottom of each tube. The bottom 5 ml of water in each tube was placed into a glass petri dish and living-inactive animals were distinguished from dead animals under a dissecting microscope. The rest of the water from each tube was also placed into petri dishs to which was added 1 ml of formalin, and the animals were then counted. Most of the animals in the samples were naupliar and early copepodid stages of *Oithona* .

RESULTS

Egg hatching rate of *Labidocera madurae*

Diel changes in UV-B radiation at each wavelength measured on July 29 are shown in Fig. 1. The daily solar ultraviolet radiation during the experiment was approximately 90% of that observed on July 28 when there was little cloud cover. Temperature during the experiment was between 26.9°C and 29.0°C. Hatching rates in the experimental (light) and control (dark) regimes were higher than 98%. No significant effect of solar UV-B radiation on *Labidocera madurae* egg hatching rate was observed (Fig. 2).

Survival rate of *Oithona* spp. nauplii

Daily solar ultraviolet radiation during the experiment is shown in Fig. 3. Solar radiation on July 28 was less influenced by clouds and the daily radiation was highest at each wavelength of UV radiation and PAR during the three experimental days. Ambient water temperature during the experiment was between 26.6°C and 29.0°C.

During the experiment, survival rates of nauplii did not decrease with time whether exposed to UV-B or not (Fig. 4). Survival rates of copepodids decreased after an incubation period longer than 48 hours. Negative influences of UV-B on survival, however, were not observed during the first two days. The contribution of *Oithona* to the total number of copepodids increased in both the experimental bottles and in the controls, and the percentage of *Oithona* to copepodids in the experimental bottles were similar to those of the two control bottles at each sampling time. Thus it may be inferred that UV-B radiation had little influence on the molting of naupliar to copepodid stages.

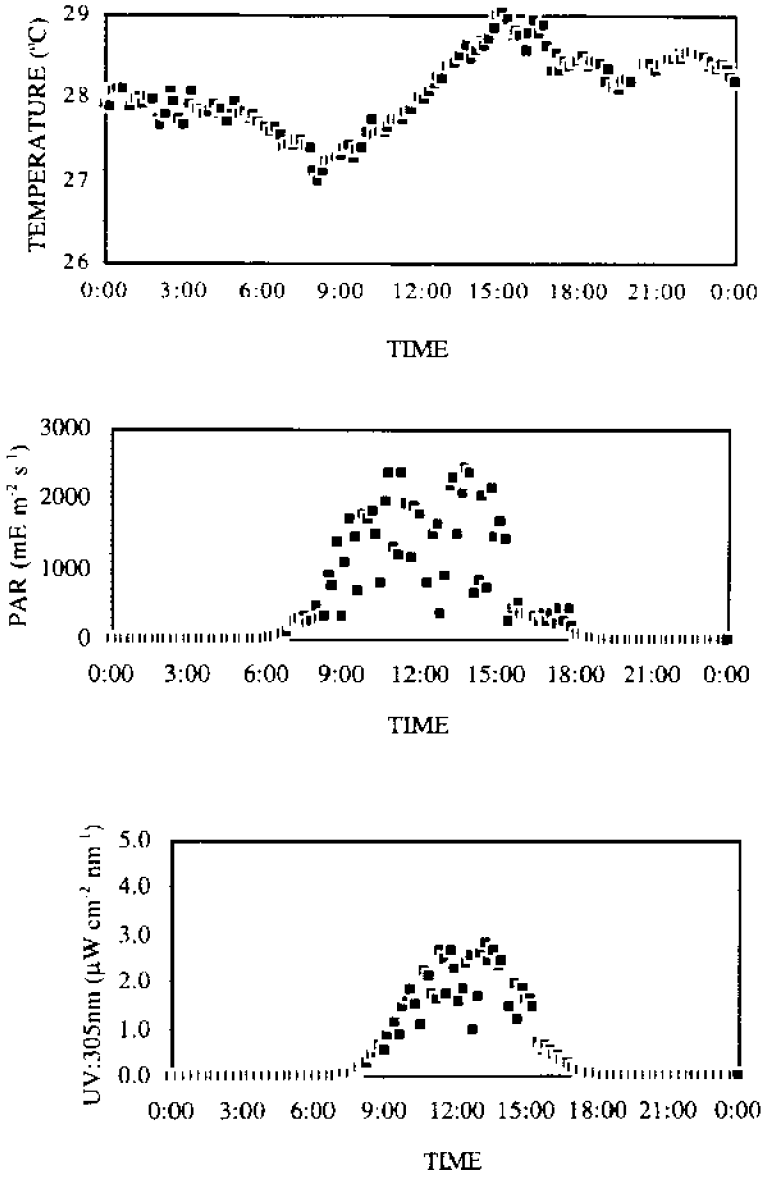

Figure 1. Diel changes in temperature, PAR, and ultraviolet radiation in wavelength of 305 nm, 320 nm, 340 nm, and 380 nm at 15 cm depth in the lagoon of Coconut Island.

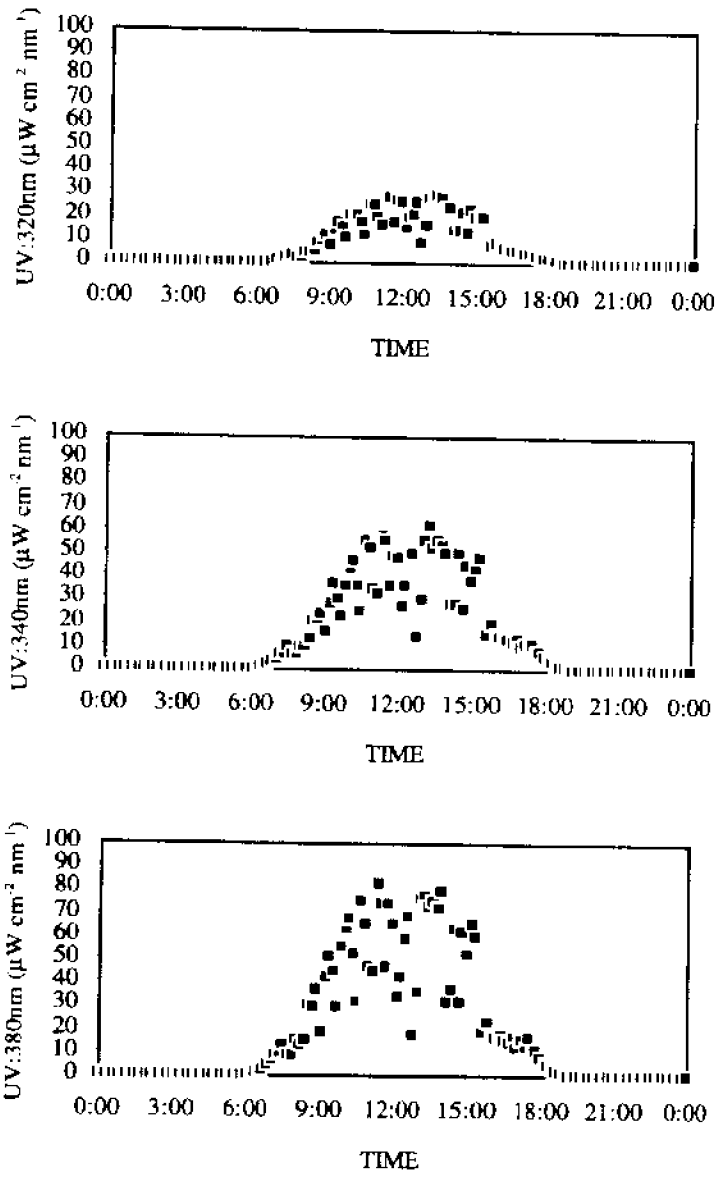

Figure 1 (cont.). Diel changes in temperature, PAR, and ultraviolet radiation in wavelength of 305 nm, 320 nm, 340 nm, and 380 nm at 15 cm depth in the lagoon at Coconut Island.

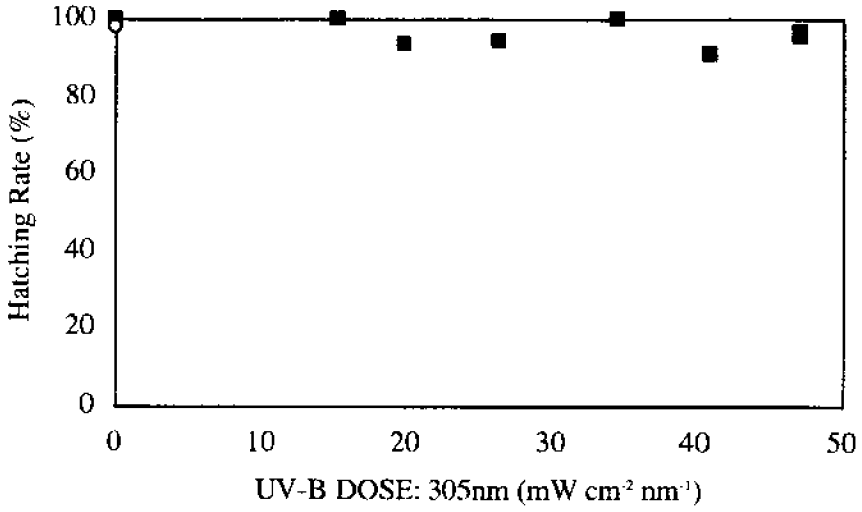

Figure 2. The influence of UV-B dose (305 nm) on the hatching rate of *Labidocera madurae*. Squares indicate experimental bottle and open circles indicate control bottle.

DISCUSSION

In this study the egg hatching rate of *Labidocera madurae* was not notably influenced by solar UV-B radiation. The daily UV radiation during the present experiment was approximately 90% of that on July 28 (Fig. 3). The daily UV radiation at the summer solstice would be higher than those observed during the present experiment. We are not sure whether solar UV radiation on a clear day around the summer solstice would influence the hatching rate of *L. madurae* or not. However, the eggs of *L. madurae* have a negative buoyancy and are adhesive. These characteristics of the eggs would suggest that the eggs may sink quickly to the bottom of the calm lagoon and become attached to the substrate. UV radiation at the bottom of the lagoon (the depth is 0.5 m to 3 m) is lower than the amounts employed in the present experiments. This suggests that naturally produced eggs are usually exposed to weaker UV at the bottom of the lagoon than the ones in the present experiments, and *in vivo* hatching may be not be influenced by UV-B.

No influence of solar UV radiation on the survival rate of *Oithona* nauplii was observed in this study. It was obvious that solar UV-B radiation did not lethally influence the naupliar stages of *Oithona* . While we did not examine the influence of UV-B on the ingestion and assimilation of food or growth rate of *Oithona* nauplii, because solar UV-B radiation had little, if any, influence on the molts of naupliar and copepodid stages, the results suggest that ingestion and growth of *Oithona* nauplii under UV-B would be similar to that of nauplii without exposure to UV-B.

In this study no influence of solar UV-B radiation was observed on copepods in the lagoon at Coconut Island. However, it is known that the present-day levels of solar UV-B radiation can cause serious damage to copepods in temperate and boreal areas. Hatching rates of the copepod *Paracalanus* sp. were decreased by solar UV-B radiation in October (Saito, unpublished data). Solar UV-B radiation in April and June lethally affected the copepod *Calanus sinicus* in the Inland Sea, Japan (Uye *et al.*, personal communication) and UV radiation in those areas is lower than that used in the present experiments. Therefore, we can say that copepods in the lagoon at Coconut Island are well-adapted to their photo-environment and they are less influenced by present levels of UV-B radiation than *Paracalanus* sp. and *Calanus sinicus*. These differences in sensitivity to UV between copepods may be due to differences in protection from UV radiation.

Forms of UV protection in copepods are not well known. Pigments and their concentration are important for protection from UV in copepods (Ringelberg *et al.*, 1984) as in the other marine

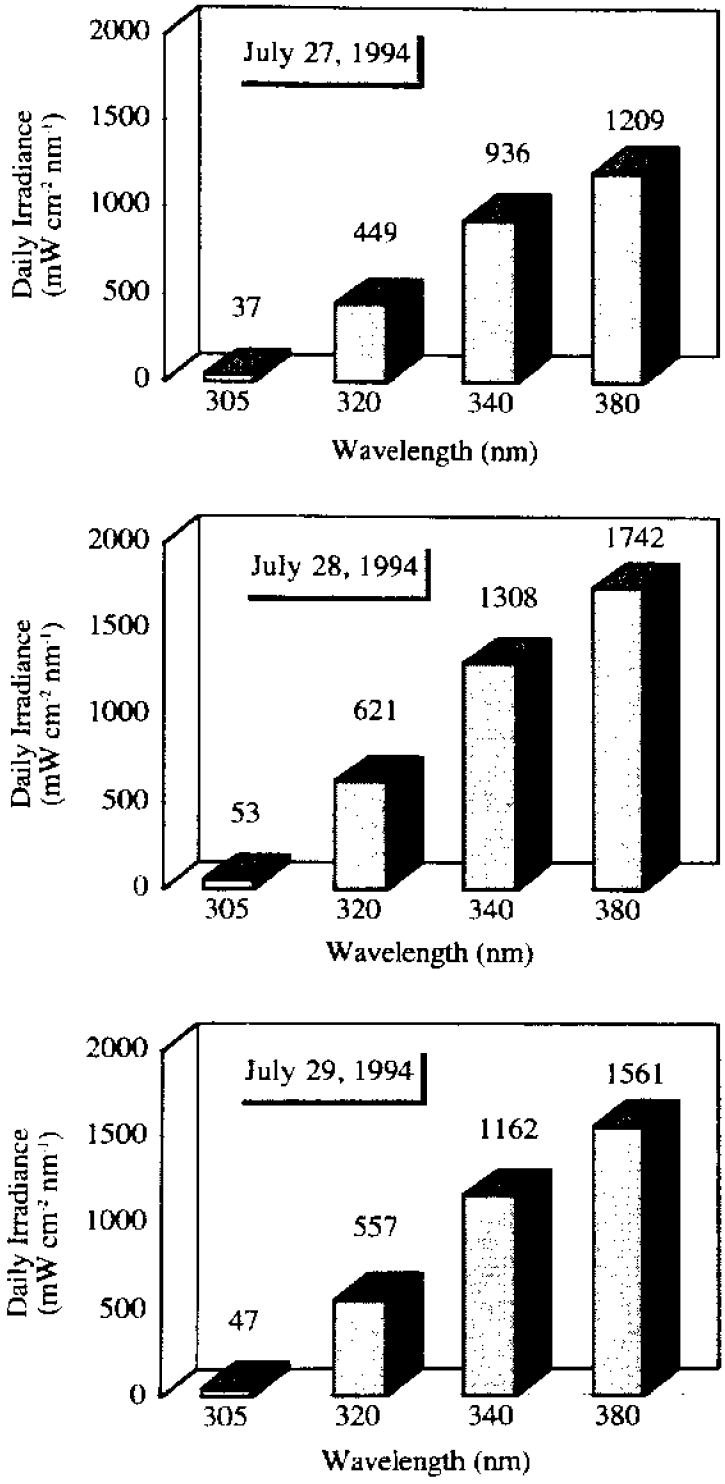

Figure 3. Daily solar ultraviolet radiation in the wavelength of 305 nm, 320 nm, 340 nm, and 380 nm at 15 cm depth in the lagoon at Coconut Island between July 27 and July 29, 1994.

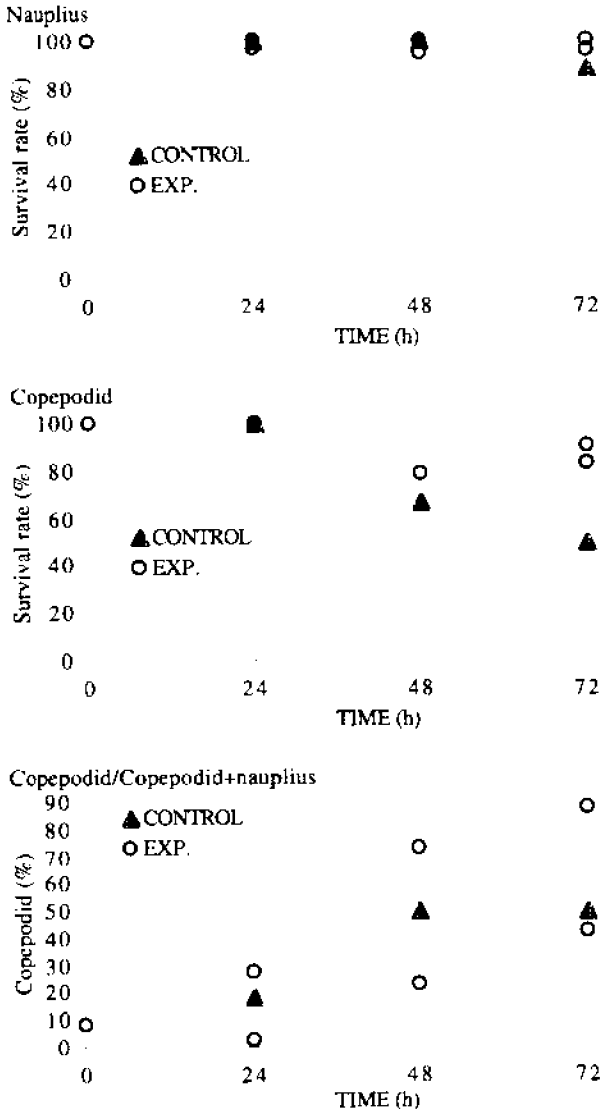

Figure 4. Influence of ultraviolet radiation on *Oithona* spp. Top and middle figures show survival rates of nauplius and copepodid stages, respectively. Bottom figure shows copepodid percentage to nauplii plus copepodids. Circles and triangles indicate experimental and control bottles, respectively.

animals (Chapman and Hardy, 1988; Shick *et al.*, 1992). Eggs of *Paracalanus* are transparent while those of *Labidocera madurae* have a green pigment. It is speculated that the green pigment in *L. madurae* eggs may block UV radiation and thus serve as one of the factors that makes *L. madurae* eggs less vulnerable to UV than eggs of *Paracalanus*.

While we have no information on UV protection in *Oithona*, the results of the present study indicate that this organism is well-protected from solar UV-B radiation. Mycosporine-like amino acids are possible candidates to serve as protective compounds in this organism as such compounds have been found in many marine organisms in the world ocean (Karentz *et al.*, 1991). Determination of such UV-protective compounds in copepods adapted to the photo-environment in the lagoon at Coconut Island should be made in the future.

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A biological weighting function for phytoplankton growth inhibition

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ABSTRACT: There is concern that reduced stratospheric ozone, and the subsequent increase in ultraviolet radiation at the surface of the ocean, could have an adverse effect on phytoplankton. Evaluating the possible influence of reduced stratospheric ozone on an organism requires an accurate estimation of the biologically weighted fluence rate, that is, the effective dose rate. The effective biological dose is the integral of the incident spectral irradiance and an organism's wavelength dependent biological response to this irradiance. Currently there is no generally accepted biological weighting function for assessing the influence of ultraviolet radiation on phytoplankton. We discuss a procedure for estimating the biological weighting function for observed growth inhibition in phytoplankton, $\varepsilon(\lambda)$, given the inhibited response of several cultures to 3 light regimes: PAR (400 - 700 nm); PAR + UV-A (320 - 400 nm); and PAR + UV-A + UV-B (280 - 320 nm). Work by Jokiel and York (1984) provides the irradiance partitions and growth inhibition data used. An atmospheric model (Green, 1980) is used to reconstitute the spectral quality of the incident irradiance. Our results show that some published biological weighting functions (DNA (Setlow, 1974), generalized plant (Caldwell, 1971), Erythema (NAS, 1979, 1982), and Cullen *et al.* (1992)) are inconsistent with computed changes in dose vs. observed changes in biological response. A functional form suggested by Rundel (1983) of $\varepsilon(\lambda) = e^{a_1 \lambda}$ was investigated. For the culture *Symbiodinium microadriaticum* an a_1 of -0.45 [nm^{-1}] produced the best fit. Although the existing data does not allow for a precise estimation of $\varepsilon(\lambda)$, the work presented here provides a method for identifying biological weighting functions which are inconsistent with the experimental results of Jokiel and York.

INTRODUCTION

The determination of an appropriate weighting function is a crucial component in the quantitative estimation of a possible ecological impact of increased UV-B, caused by decreased stratospheric ozone, on phytoplankton (NAS, 1979, 1982, 1984; Rundel, 1983; Caldwell et al., 1986). Biological weighting functions (also called "action spectra") are used, along with spectral irradiance, in calculating biologically weighted fluence rate (or "dose").

$$E_B = \int \varepsilon(\lambda)\, E(\lambda)\, d\lambda \qquad (1)$$

where $\varepsilon(\lambda)$ is the wavelength dependent action spectrum and $E(\lambda)$ is the spectral irradiance. Representative spectral curves are shown in Fig. 1. Biological fluence rates can be especially sensitive to changes in the spectral irradiance particularly when the wavelength dependent slopes of $\varepsilon(\lambda)$ and $E(\lambda)$ are opposed.

All the action spectra considered were normalized at 290 nm, i.e., $\varepsilon(\lambda)_{normalized} = \varepsilon(\lambda)/\varepsilon(290\ nm)$. For the subsequent discussion the subscript "normalized" is assumed and this notation dropped.

The spectral shape of $\varepsilon(\lambda)$ will greatly influence the potential effects of ozone diminution on phytoplankton productivity. It is possible for a relatively large increase in middle ultraviolet (UV-B 280 - 320 nm) radiation to cause only a small fractional increase in the biological fluence rate. This could be true if the action spectrum for a biological effect on phytoplankton $\varepsilon(\lambda)$, were evenly weighted across the ultraviolet and visible wavelengths or had a peak somewhere in the visible. In this case, the potential for ozone diminution to effect phytoplankton productivity would be small. Conversely, if $\varepsilon(\lambda)$ is highly weighted in the near and middle ultraviolet, the potential for effects on phytoplankton productivity could be significant. Conclusive evidence of reduced algal productivity due to UV-B exposure has been demonstrated by many investigators (Worrest et al., 1978, 1980, 1981; Smith and Baker, 1980; Worrest, 1982, 1983; Haeder, 1984, 1985, 1986,

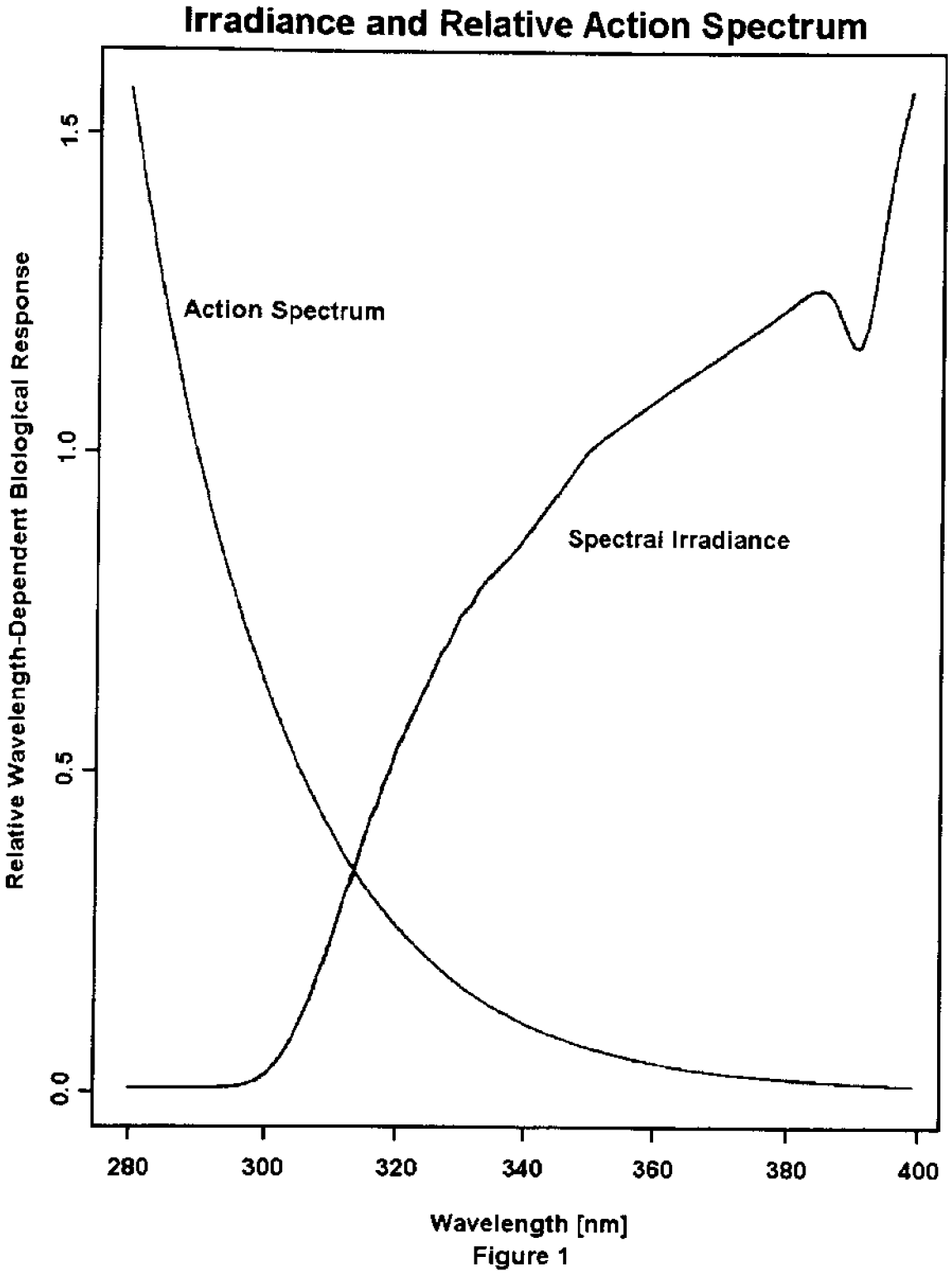


Figure 1. Schematic of spectral irradiance at the earth's surface compared with an action spectrum to illustrate opposing behavior as a function of wavelength.

1987; Haeder and Haeder, 1988; Smith et al., 1992). UV-B penetrates natural waters to ecologically significant depths (Jerlov, 1950; Calkins, 1975; Smith and Baker, 1979, 1981) so there is a potential to adversely influence phytoplankton productivity. The estimation of dose as a function of depth (Smith and Baker, 1979) is strongly dependent on the spectral shape of $\varepsilon(\lambda)$.

There are several potential action spectra which have been suggested as biological weighting functions for describing the effects of enhanced UV-B on phytoplankton productivity. In spite of the importance of accurate action spectra, there is considerable uncertainty with respect to the primary target for inhibition and the corresponding $\varepsilon(\lambda)$. Smith (1989) and Smith and Baker (1979) have reviewed the problems associated with these uncertainties. These authors point out that usual ^{14}C techniques for estimating phytoplankton productivity in which relatively short-term incubations provide an estimate for the biological weighting for photoinhibition $\varepsilon_{ii}(\lambda)$, may be inadequate for assessment of possible longer term UV-B damage that may be ecologically significant.

As an alternative to ^{14}C productivity experiments, Jokiel and York (1984) carried out long term (1-2 weeks) monoculture experiments with natural sunlight and combinations of UV filters and neutral density filters to determine the relative importance of present day levels of solar UV radiation and PAR in causing microalgae growth inhibition. These workers found that long-term growth photoinhibition (when observed) was "due almost entirely to UV radiation." They used various radiation regimes which included PAR (400 - 700 nm), UV-A (320 - 400 nm) and UV-B (280 - 320 nm) and natural solar radiation was filtered into 3 light regimes; PAR, PAR + UV-A, and PAR + UV-A + UV-B. Each regime was then reduced to 92%, 38%, 20% and 6% of its intensity using neutral density filters, giving twelve radiation treatments for each experiment. We use their data in the following.

MATERIALS AND METHODS

Abridged spectrophotometry (Nachtwey and Caldwell, 1975; Smith and Baker, 1982) is a method which can be used for estimating $\varepsilon(\lambda)$. This involves;

1) hypothesizing a possible action spectrum;
2) calculating dose rates for the several treatments using this action spectrum;
3) plotting the computed dose rates for this hypothesized $\varepsilon(\lambda)$ vs. the observed growth inhibition and
4) checking these results for internal consistency.

If the trial $\varepsilon(\lambda)$ has the correct spectral weighting, then all the data points would fall along a curve describing the organism's response to varying dose rates. Using this technique, we tested several published action spectra (Figure 2); DNA (Setlow, 1974), generalized plant (Caldwell, 1971), Erythema (NAS, 1979, 1982), Photoinhibition (Jones and Kok, 1966; Smith *et al.*, 1980), and Cullen *et al.*, (1992). As discussed below, none of these biological weighting functions produced results consistent with Jokiel and York's (1984) data. In contrast, reasonably consistent agreement was achieved by generating an action spectrum of the form $e^{a_1\lambda}$ as suggested by Rundel (1983). To test the sensitivity of the methods, we also investigated action spectra of the forms $\lambda^N e^{a_1\lambda}$ and $\lambda^N e^{a_1\lambda^2}$ N = 0,1,2,3 and a_1 varying so as to produce a wide range of results.

By making use of the relationship (Rundel, 1983) between differential biological response and differential biological effective dose,

$$\Delta E_{BE} = \int \Delta E(\lambda)\, \varepsilon(\lambda)\, d\lambda \qquad (2)$$

it is possible to associate observed fractional growth reduction with the corresponding change in dose rate. Jokiel and York (1984) observed that only UV-A and UV-B caused long-term growth inhibition, so we took the growth rate for PAR at a particular intensity to be our baseline. The fractional growth reduction from this value for a given treatment is plotted against the difference in calculated dose. No assumption is made *a priori* about the shape of this curve; all that is required is a relative agreement for the effects of the various light regimes consistent with the $\varepsilon(\lambda)$ being tested.

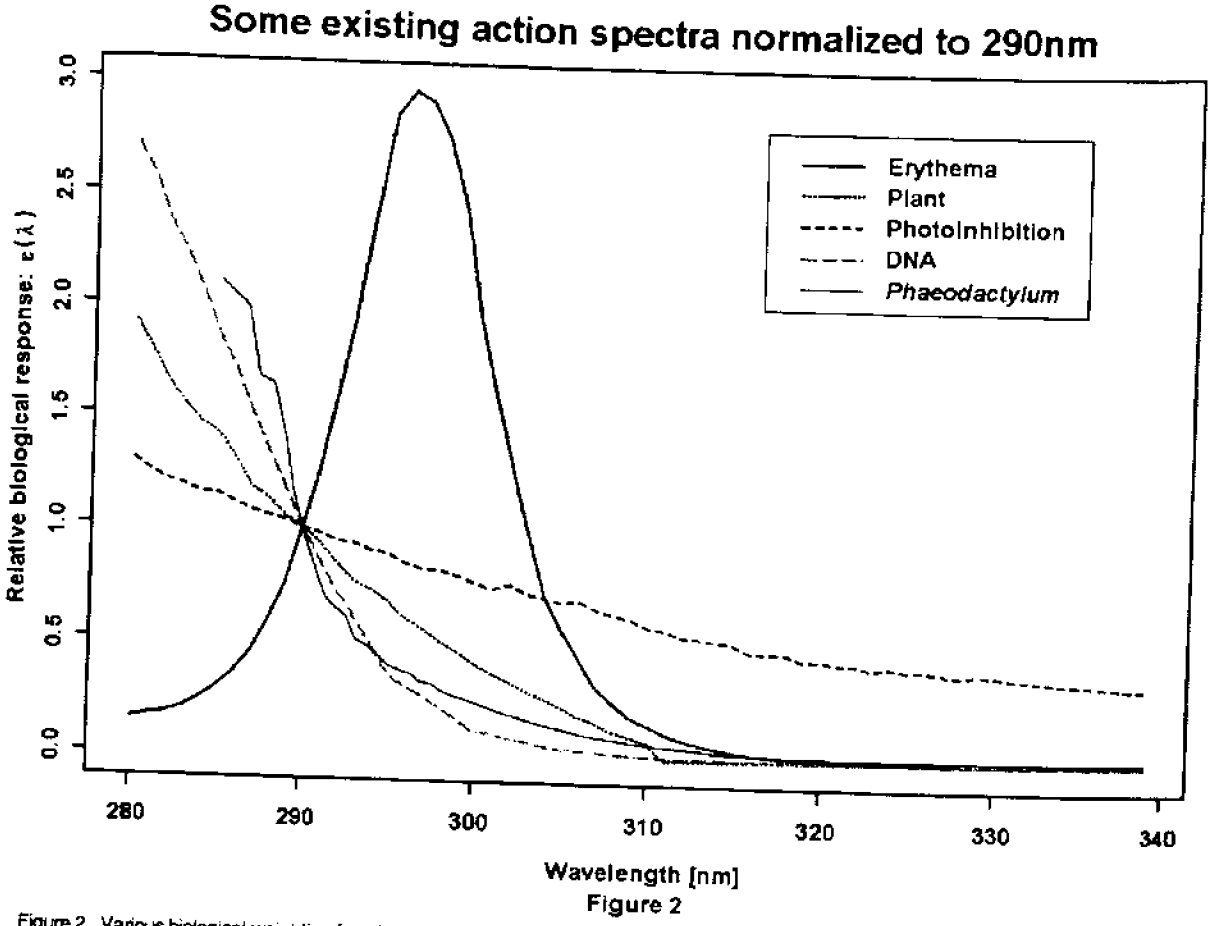


Figure 2

Figure 2. Various biological weighting functions, normalized to 290nm, versus wavelength: Generalized plant (Caldwell, 1971), DNA (Setlow, 1974), Photoinhibition (Jones & Kok, 1966), Erythema (NAS, 1979, 1982), *Phaeodactylum* (Cullen *et al.*, 1992).

The equipment necessary to accurately determine the spectral irradiance in the ultraviolet portion of the spectrum was not available during these experiments. As a consequence, surface irradiance values were estimated using an atmospheric model (Green *et al.*, 1980) with atmospheric parameters chosen to match the location and time of Jokiel and York's work. The model allows a good first order estimation of the incident spectral irradiance, and provides an accurate estimate of the spectral shape in the ultraviolet region of the spectrum. Our absolute dose rates will be in error, but the relative values are accurate and suitable for this technique of abridged spectrophotometry.

DISCUSSION

We found the "best fit" of observed growth inhibition to calculated dose rates was

$$\varepsilon(\lambda) = e^{-0.45\lambda} \qquad (3)$$

(where λ is the wavelength in nanometers). Figure 3a,b,c shows the steps involved in formulating this conclusion. In Figure 3a, the relative action spectrum is shown with respect to the various

filtered radiation regimes. The various irradiance regimes are products of the incident solar irradiance and the transmittance of the filters [$E_{rr}(\lambda) = E_{solar}(\lambda)\ T(\lambda)$]. We show data from 280 - 450 nm since for $\lambda < 280$ nm $E_{solar}(\lambda)$ is insignificant and for $\lambda > 450$ nm $\varepsilon(\lambda)$ is insignificant. Figure 3b shows the integrand of Eq. (1), and the biologically weighted fluence rate as a function of wavelength (spectral dose vs. wavelength). Figure 3c is a plot of the observed fractional growth reduction versus changes in dose for the four neutral density filter treatments in the PAR + UV-A + UV-B light regimes. $E(\lambda)$ is reduced through the use of neutral density filters which changes the magnitude of the dose, calculated as shown in Figure 3b. The difference in dose due to the use of neutral density filters is what is being referred to as "change in dose" on Figure 3c and in later figures.

In contrast to the relative agreement shown in Figure 3c, the results for non-consistent action spectra; a) $\varepsilon_{Erythema}$, b) ε_{Plant}, c) ε_{DNA} and d) $\varepsilon_{Phaeodactylum}$ are shown in Figures 4 and 5. The divergence in Figure 5 between the calculated dose rates for UV-A (1's) and UV-B (2's) give a clear indication that the action spectra used does not represent an accurate description of the biological weighted response to spectral irradiance. These four action spectra are insufficiently weighted in the UV-A region to agree with the results of Jokiel and York (1984).

There are at least two reasons why the dose versus effect plots are inconsistent: first, the weighting function under test is incorrect for the biological effect studied; second, the assumption of reciprocity (i.e., dose is the product of intensity times time) may not hold for the range of doses given in the experiment. We have no information with respect to dose versus response for the growth inhibition observed here. Assuming reciprocity holds, we conclude that biological weighting functions like $\varepsilon_{UV\text{-}A}$, which shows a relatively high weighting in the UV-A region of the spectrum, is required for consistency with the long-term growth inhibition results observed by Jokiel and York (1984). If these results prove to be of general validity with respect to UV effects on natural phytoplankton populations, then the predicted influence of enhanced UV-B on these populations will be less than that predicted for ε_{DNA} but greater that that for $\varepsilon_{Photoinhibition}$.

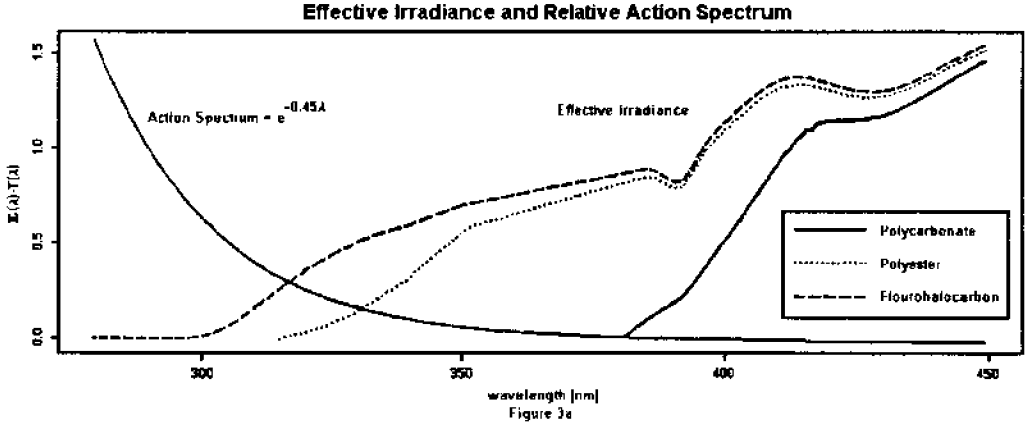


Figure 3a. Incident spectral irradiance for several radiation regimes and the UV-A (Rundel) action spectrum versus wavelength.

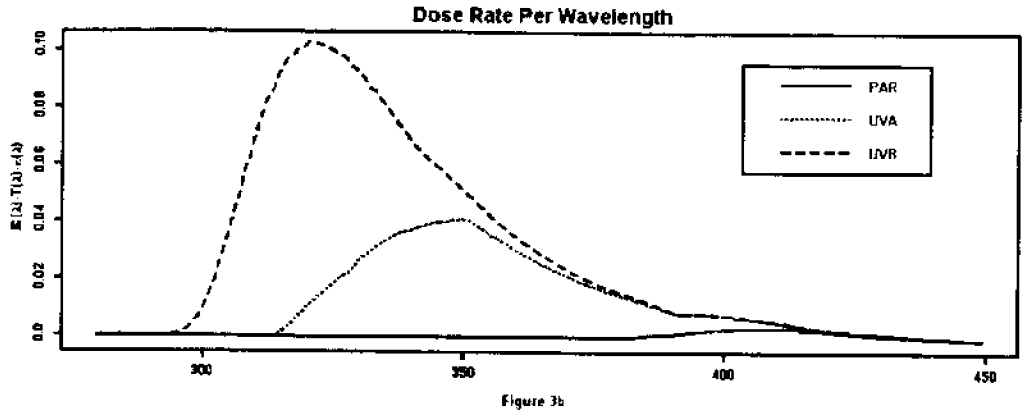


Figure 3b. Biologically weighted fluence rate (dose) versus wavelength for the radiation regimes shown in Fig. 3a.

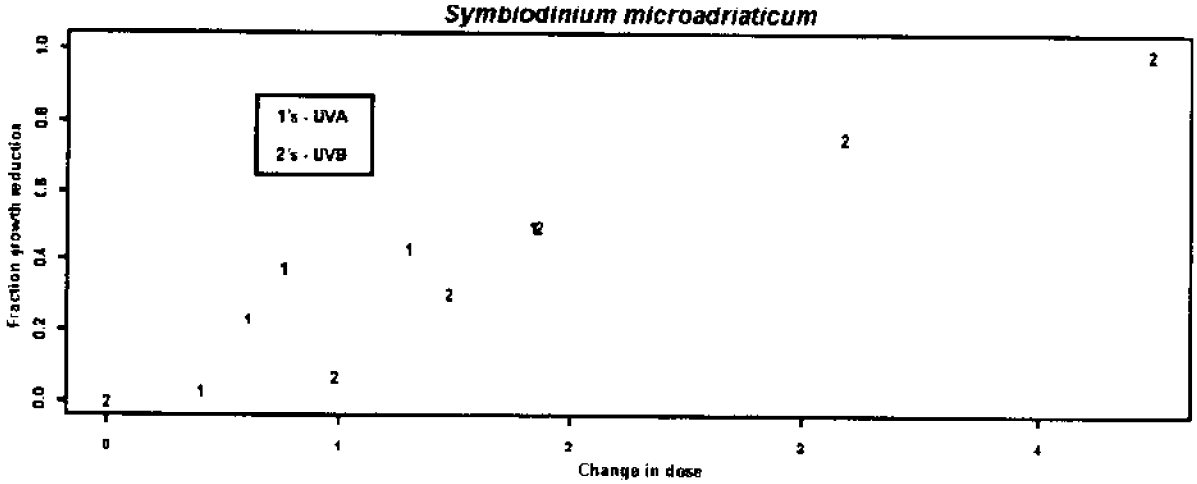
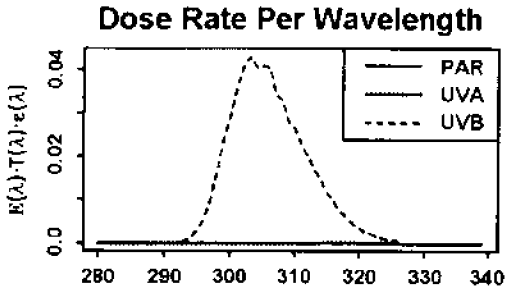
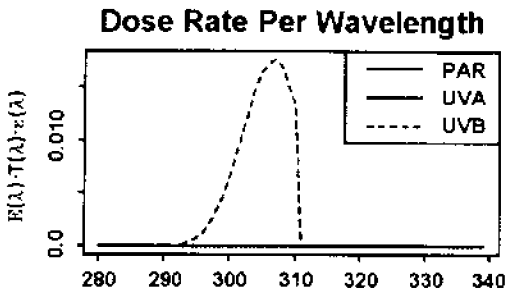


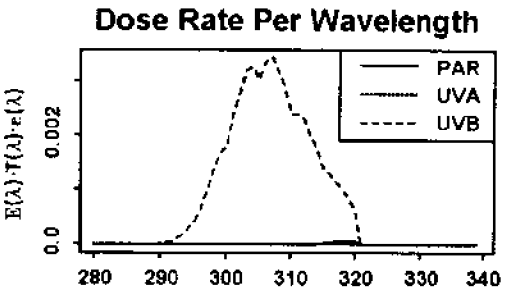
Figure 3c. Fractional growth reduction versus change in biological dose for the UV-A radiation regime (1's) and the UV-B radiation regime (2's).



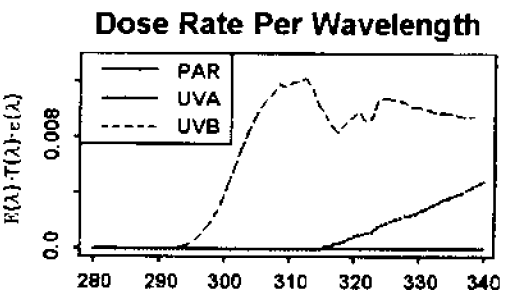
Erythema Action Spectrum
Figure 4a



Plant (Caldwell) Action Spectrum
Figure 4b



DNA Action Spectrum
Figure 4c



Phaeodactylum Action Spectrum
Figure 4d

Figure 4. Biological weighted fluence versus wavelength for several radiation regimes and for various action spectra.

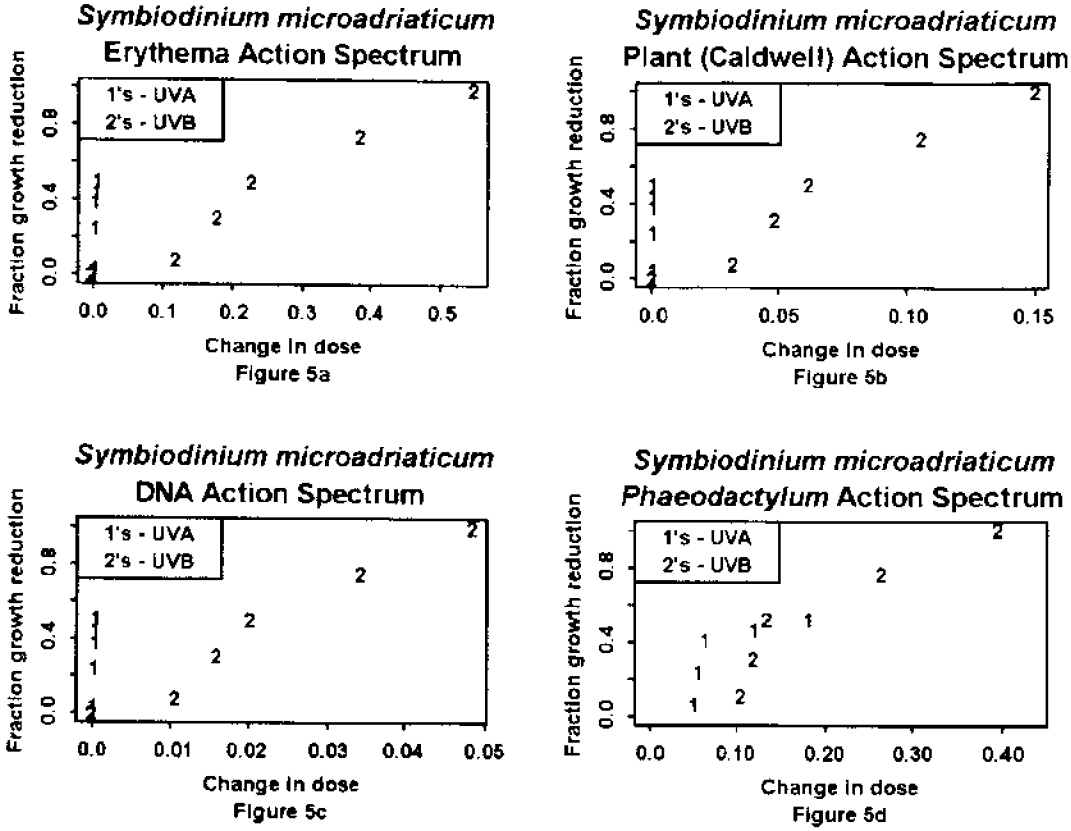


Figure 5. Response versus dose for UV-A (1's) and UV-B (2's) radiation regimes for various action spectra.

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Physiological and biochemical effects of UV radiation on the marine phytoplankton *Nannochloropsis* sp. and *Dunaliella* sp.

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ABSTRACT: The effects of UV-A and UV-B radiation on photosynthetic parameters and antioxidative mechanisms in *Nannochloropsis* sp. and *Dunaliella* sp. were investigated. Both UV-A and UV-B radiation caused decreased growth rates in *Nannochloropsis* but did not significantly effect *Dunaliella* growth. Photosynthesis in *Nannochloropsis* was adversely affected primarily by UV-B as reflected in the reduction of Rubisco and LHCPII levels. After 48-hours, photosynthetic parameters such as light utilization efficiency and quantum utilization recovered from the initial shock of exposure to UV radiation. In addition, there was a decrease in stress-responding antioxidative enzymes and corresponding increases in Rubisco and LHCPII levels. UV-B caused a significant increase in both Mn SOD, CuZn SOD and catalase activities after 4-hours. Overall, *Dunaliella* was less sensitive to UV stress. Possibly this was due to a well-developed continuous cell repair mechanism which prevented UV-induced damage under these experimental conditions.

Erratum:

Page 61, paragraph 1, line 8 "animals" should be "plants".

INTRODUCTION

Current concern over the world-wide effects of solar ultraviolet (UV) radiation has led to biological investigations spanning the molecular to community levels of ecological organization. As water covers about two-thirds of the earth's surface, the importance of understanding and predicting the influence of UV-A and UV-B radiation on both freshwater and marine environments cannot be underestimated, especially in view of their high penetration in seawater (Smith *et al.*, 1992). Phytoplankton, an integral component in the biogeochemical cycling process, are susceptible to photochemical damage due to UV radiation (Jokiel, 1984). Any profound effect on this group of ~~animals~~ could possibly cause an imbalance in global supplies of photosynthetically fixed carbon and disruption of trophic dynamics.

Oxidative stress and photosynthesis are intrinsically interconnected. An oxidant is produced as a result of illumination. Suboptimal conditions for photosynthesis, such as high light intensity or low CO_2 concentrations, cause a decrease in the efficiency of energy transfer from photons to CO_2 and result in damage to the chloroplasts. Photosensitizers, such as chlorophyll and flavins, absorb the UV wavelengths of solar irradiance, and form reactive molecules such as triplet excited chlorophyll and active oxygen species (e.g., $O_2^{\cdot}$, H_2O_2, and $OH^{\cdot}$) when excess light energy is not dissipated as heat or fluorescence(Asada & Takahashi, 1987; Asada, 1994). Furthermore, these active oxygen species can oxidize membrane lipids and proteins, and cause general cell destruction (Fridovich, 1986).

Both plants and algae possess complex antioxidative mechanisms which counter the adverse effects of oxygen radicals. These mechanisms buffer energy transfer limitations which would ultimately lower photosynthetic activity and prevent deleterious oxidation reactions within the cells. The superoxide ($O_2^{\cdot}$) scavenging metallo-enzyme, superoxide dismutase (SOD), exits in three main forms: CuZn SOD(chloroplastic and cytostolic), Mn SOD(mitochondrial) and Fe SOD. The dismutation of $O_2^{\cdot}$ to H_2O_2 is rapidly followed by inactivation of this product by ascorbate peroxidase or catalase by reduction or disproportionation respectively (Asada, 1994).

Recent research on endogenous protective mechanisms in the zooxanthellae of *Aiptasia pallida*, demonstrated significantly increased Super Oxide Dismutase (SOD) and catalase activities after exposure to UV radiation (Lesser *et al.*, 1989). *Chaetoceros gracilis*, a marine diatom,was similarly exposed to UV irradiation and while there was no significant change in SOD activity over 48-hours, catalase activity did increase on acclimation to PAR + UV (Hazzard, 1994), probably due to an increase in UV-induced oxygen radical production.

In this research we aimed to elucidate the nature of defined UV-induced changes in SOD and catalase activitiy in different size marine phytoplankton. The relevance of species and size differences was illustrated by Karentz *et al.* (1991) who showed differential cell survival characteristics and photo-enhanced repair under conditions of UV irradiation. Therefore it is relevant to investigate the effects of different UV wavelengths on defense mechanisms which

could affect repair mechanisms in different species. In addition, the site of the specific UV-induced changes is indicated by the relative changes in CuZn SOD and Mn SOD. In order to gain an insight into the effects of UV-A and UV-B radiation on photosynthesis, we established Photosynthesis vs Irradiance (P vs I) curves which provided information on the physiological status of the cells and the potential effects of vertical mixing in the water column.

To date, most of the work on photosynthesis has concentrated on the light intensity-dependent regulation of ribulose-1,5-bis phosphate carboxylase-oxygenase (Rubisco) and the light harvesting pigment protein complex of photosystem II (LHCPII) (Morttain-Bertrand *et al.*, 1990; Falkowski & LaRoche, 1991). Rubisco is the main enzyme participating in photosynthetic carbon fixation and LHCPII is the main protein connecting the light harvesting pigments of photosystem II (PSII). A positive correlation between photosynthetic parameters and Rubisco was reported by Falkowski and Laroce (1991). Also, changes in the amount of LHCPII and Rubisco were found during acclimatization to both high and low light levels. It was concluded that such modifications in each protein pool play an important role in algal cells acclimating to changes in ambient light regimes. Few reports have examined changes in Rubisco and LHCPII levels under UV-A and UV-B stress, so this work also gives an initial insight into molecular photo-acclimation due to UV radiation stress.

MATERIALS AND METHODS

Experimental set-up

Cultures of the marine phytoplankton *Nannochloropsis* sp. (Eustigmatophycae) and *Dunaliella* sp. (Chlorophycae) were obtained from the culture collection of the Hawai'i Institute of Marine Biology (HIMB) and grown under Westinghouse "Cool White" fluorescent lights. The cultures were inoculated in nine liters of F2 medium in order to obtain cells in a logarithmic growth phase prior to day 1 of the experiment. Duplicate flasks of cultures (for each time period) were incubated in an outdoor water bath and exposed to three different irradiation treatments:

Treatment	Symbol	Filter
Full Solar Spectrum (UV-B + UV-A + PAR)	(UVT)	Aclar® 33c Fluoropolymer film
No UV-B (UV-A + PAR)	(UVA)	Mylar® Type D Fluoropolymer film
No UV (PAR)	(UVO)	100% Clear Acrylic Safety Glazing sheet

The cells were analyzed after 4-, 24- and 48-hours exposure to the described conditions. The natural irradiation was plotted during the experiments to insure that there were no major differences in radiation dosage throughout the 3-day experiments. Figure 1 shows a typical 24-hour radiation profile as measured and recorded at the weather station located at the Point Laboratory, HIMB (See Gulko *et al.*, this volume).

Cell number and chlorophyll

At each time period a sample of each duplicate treatment was fixed in Lugol's solution and the cell number was determined using a hemocytometer counting chamber and a light microscope. Chlorophyll *a* and carotene were calculated for each treatment after filtration on a GFC filter and overnight extraction in 90% methanol (Jeffrey & Humphrey, 1973).

Photosynthetic response

A Photosynthesis vs Irradiance (P vs I) curve was established for each of the treatments using a YSI oxygen microelectrode which measured evolution of oxygen during photosynthesis. The oxygen released was converted into an electronic signal and the data was collected using an analog system. Photosynthesis was then calculated per unit cell for chlorophyll (Dubinsky *et al.*, 1987). The photosynthetic parameters were calculated according to Fisher (1987) and included derivation of the optical cross section. The optical cross section is defined as the *in vivo*, spectral average, chlorophyll *a*-specific absorption cross section (a^*) as calculated from each culture's absorbance and chlorophyll *a* concentration (Fisher, 1987).

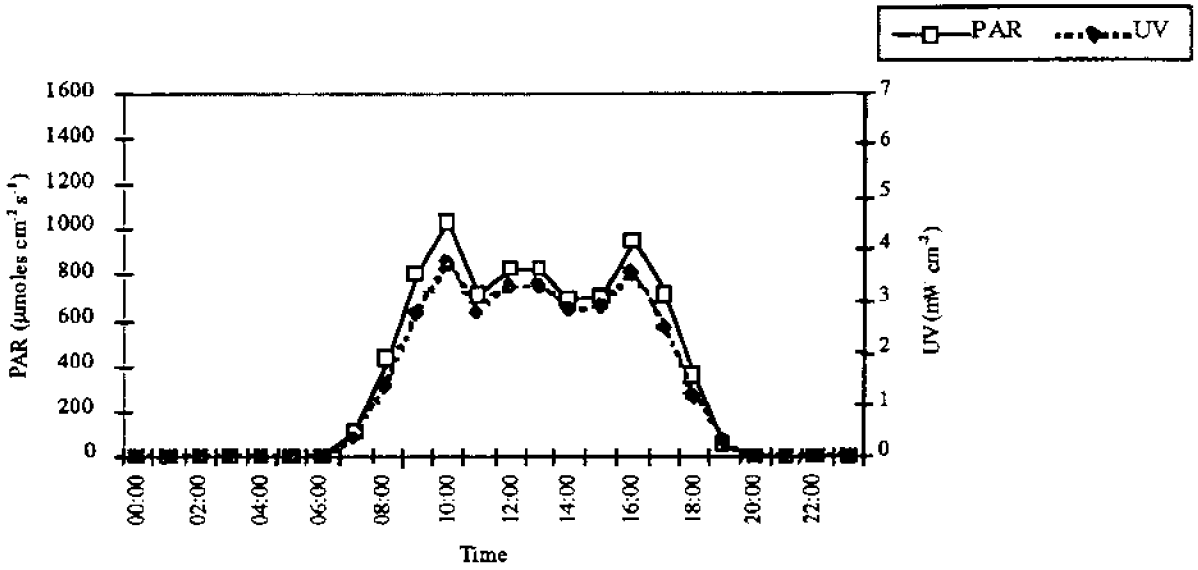

Figure 1. A typical natural surface radiation environment profile over a 24 hour period showing photosynthetically active radiation (PAR) and total ultraviolet radiation (UV <400 nm).

Antioxidative enzyme activity

The cells were concentrated by centrifugation at 5,000 rpm and homogenates were prepared using glass beads and a Teflon® homogenizer. The resulting suspension was spun down at 14,000 rpm and the supernatant used as the crude enzyme extract after filtration on a 0.45 µm Millipore® filter.

Catalase activity was measured spectrophotometrically according to Beers and Sizer (1952). CuZn SOD and Mn SOD activities were quantified by a modified end-point method (Oyanagui, 1984). Total SOD was assayed in the absence of KCN whereas Mn SOD was measured in the presence of 3 mM potassium cyanide. CuZn SOD (KCN-sensitive) was calculated as the difference between total SOD and Mn SOD. Total protein was estimated according to Bradford (1976).

Rubisco and LHCPII content

The homogenate described above was further purified according to Sukenik *et al.*, (1992). The resulting protein extract was analyzed for Rubisco and LHCPII content using sodium dodecyl sulfate (SDS) polyacrylamide-gel electrophoresis (PAGE) described by Laemmli (1970). After separation, 10 µg protein was loaded onto each lane and the gel was stained with Coomassie Brilliant Blue. The positions of the Rubisco and LHCPII proteins were verified by comparison with peroxidase-stained gels which were reacted with antibodies to Rubisco and LHCPII using the

Western blot procedure. Polyclonal antibodies against LHCPII and Rubisco from *Nannochloropsis* were kindly supplied by Dr. A. Livne, I.O.I.R., Haifa, Israel. A densitometer scanner was used to calculate the amounts of Rubisco (large and small subunit) and LHCPII according to their area on the gel. Following their staining, the gels were photographed using a video camera. Before the optical density was measured (Image Pro Plus® program), the gel was calibrated against the background using increasing concentrations of protein. Values presented are averages of duplicate samples and were calculated as % area/10 µg protein; the relative amounts of Rubisco and LHCPII were determined for each treatment. The Lowry method (1956) was used to determine protein concentration.

RESULTS

The cloudy nature of the weather in Hawai'i caused fluctuations in the radiation profiles, but the overall radiation intensity (PAR + UV) did not vary significantly during the course of the two, 3-day experiments. Midday PAR values, however, varied from 800-1400 µmol photons s^{-1} m^{-2} and UV values varied from 3-6 mW cm^{-2}. The pattern of incident UV radiation was always parallel to that of PAR alone .

Cell number and chlorophyll

Both *Nannochloropsis* and *Dunaliella* showed an increase in cell number during the course of each 3-day experiment (Figs. 2a and 2b). *Dunaliella* showed little difference in growth between the three radiation treatments although at the end of 48-hours, both UVA and UVT gave a slightly higher cell number. Conversely, *Nannochloropsis* showed a well-defined response to PAR radiation only; the number of cells was higher at 24-and 48-hours than under UVT or UVA treatments.

Chlorophyll *a* content of *Nannochloropsis* decreased on exposure to all radiation treatments (except initially to UVO). This was also true for *Dunaliella* although there were signs of a recovery in chlorophyll content after 48-hours. This decrease may have been due to the sudden exposure to natural high irradiances as compared to the low laboratory lighting. The ratio of carotene to chlorophyll *a* increased with time for all treatments for *Nannochloropsis*, while this value stayed constant throughout the experiment for *Dunaliella* .

Photosynthetic parameters

Typical hyperbolic curves were obtained when the photosynthetic potential of the two species was measured under increasing irradiation intensities; representative P vs I curves for *Nannochloropsis* (after 4-hour exposure) and *Dunaliella* (after 24-hour exposure) are shown in Figs. 3 and 4. Photosynthesis increased up to a saturating irradiation intensity (I_k) between 60-240 µmols photons $m^{-2}s^{-1}$, and then either remained stable or decreased with further increases in irradiation intensity. There was a decrease in P_{max} when exposed to UVT for both *Nannochloropsis* and *Dunaliella* on a per unit chlorophyll and per cell basis. In *Nannochloropsis*, P_{max} per unit chlorophyll increased under a UVA regime; the opposite was true for *Dunaliella*, where UVA inhibited photosynthesis more than UVT.

Details of the effects of UV radiation on different photosynthetic parameters are shown in Tables I and II for *Nannochloropsis* and *Dunaliella*, respectively. On a per cell basis, the radiation utilization efficiency of *Nannochloropsis* decreased steadily after 24-hours of exposure to UV-A and UV-B radiation, as compared to PAR only. UV-A + PAR resulted in a less dramatic decrease in photosynthetic efficiency after 24-hours. After 48-hours of exposure, all *Nannochloropsis* samples showed similar photosynthetic efficiencies (per cell). The UVT treatment also adversely affected the optical cross section (a*) after only 4-hours, but this "recovered" after 24-hours. The maximal quantum utilization for both UVT and UVA treatments were both much higher than the control (UVO) value after 4-hours of exposure. However, 24-hours of exposure caused a great decline in the quantum utilization and by 48-hours, cells exposed to UVT gave only half the value of cells exposed to UVO.

Unlike *Nannochloropsis*, the photosynthetic efficiency of *Dunaliella* increased after 4-hours under UVT and UVA treatments compared to PAR-only cells. This situation was reversed after 48-hours. The a* values for *Dunaliella* cells exposed to UVT and UVA were greatly reduced after 4-hours relative to the UVO treatment. After 48-hours, values were more comparable with UVT-treated cells having the highest optical cross section. Quantum utilization values for *Dunaliella* show that UVT caused an immediate decline relative to UVO treatment and that this effect was sustained throughout the 48-hour treatment. Although no clear pattern was shown, there was a general increase in respiration after 4- and 24-hours for both algae, which then decreased after 48-hours.

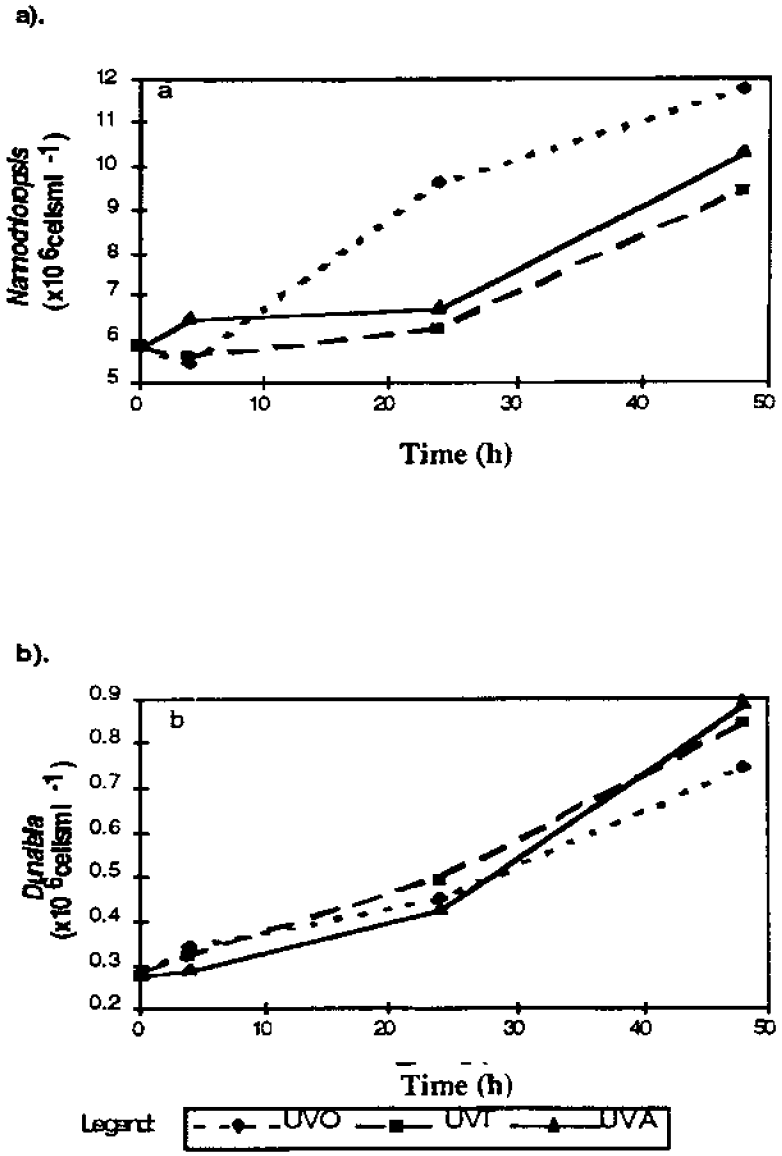

Figure 2. Changes in cell numbers over a 48-hour period of exposure to UVO (PAR only), UVT (PAR+UV-B+UV-A) and UVA (PAR+UV-A) for a) *Nannochloropsis* and b) *Dunaliella*.

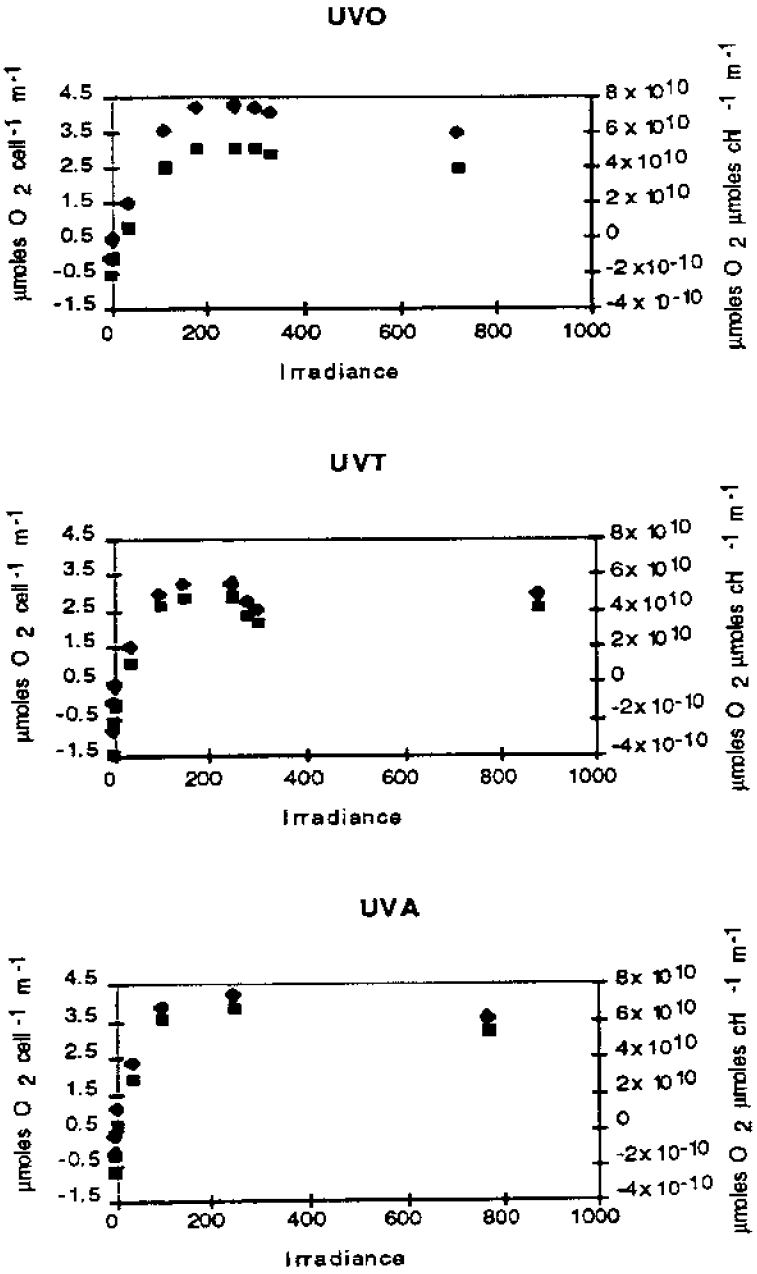

Figure 3. Photosynthesis vs. Irradiance curve after 4h exposure for *Nannochloropsis*. Photosynthesis expressed per unit chlorophyll (B) and per cell (F). Irradiance units=µmol photons m^{-2} s^{-1}.

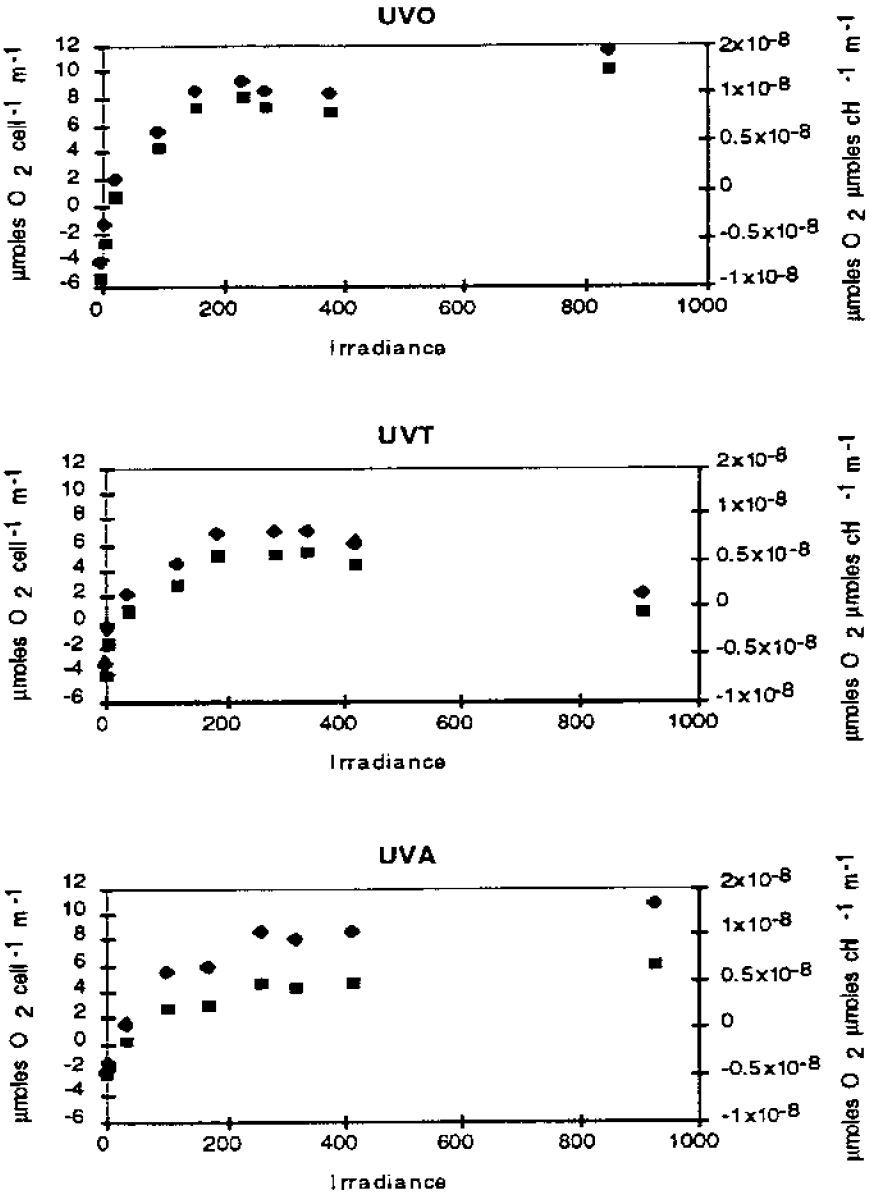

Figure 4. Photosynthesis vs. Irradiance curve after 24h exposure for *Dunaliella*. Legend as in Fig. 3.

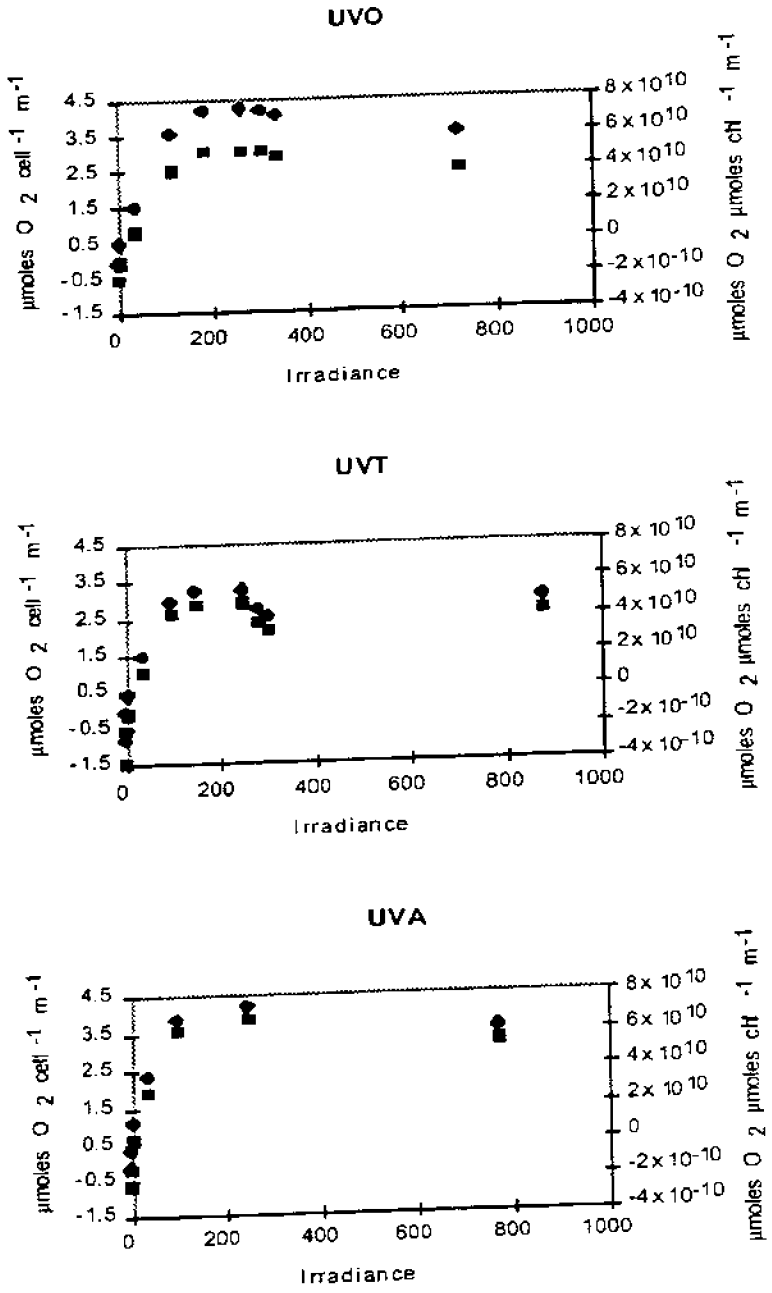

Figure 3. Photosynthesis vs. Irradiance curve after 4h exposure for *Nannochloropsis*. Photosynthesis expressed per unit chlorophyll (■) and per cell (●). Irradiance units=µmol photons m^{-2} s^{-1}.

Table I. The effect of different UV regimes on growth parameters.

	t=0	4h UVO	4h UVT	4h UVA	24h UVO	24h UVT	24h UVA	48h UVO	48h UVT	48h UVA
Nannochloropsis										
pg chl cell^{-1}	0.12	0.16	0.1	0.1	0.06	0.06	0.07	0.07	0.06	0.05
pg carotene cell^{-1}	0.04	0.06	0.03	0.03	0.02	0.03	0.03	0.03	0.03	0.02
carotene chl^{-1}	0.32	0.35	0.31	0.33	0.4	0.41	0.44	0.46	0.48	0.45
cells x 10^{6} ml^{-1}	5.8	5.43	5.67	6.22	9.59	6.23	6.68	11.7	9.43	10.3
Dunaliella										
pg chl cell^{-1}	2.36	1.4	1.71	1.82	1.26	1.62	145	1.9	1.76	1.58
pg carotene cell^{-1}	0.95	0.57	0.69	0.74	0.5	0.65	0.58	0.76	0.71	0.63
carotene chl^{-1}	0.4	0.4	0.4	0.4	0.4	0.4	1.4	0.4	0.4	0.4
cells x 10^{6} ml^{-1}	0.275	0.338	0.322	0.285	0.446	0.493	0.426	0.741	0.841	0.885

Table II. Effect of radiation on photosynthetic parameters for *Nannochloropsis*.

Time (hous)	Treatment	α (x10^{-4} chl^{-1})	α (x10^{-11} cel^{-1})	a*	ϕ	I/ϕ	P max. Net (x10^{-10} chl^{-1})	P max. Gross (x10^{-10} chl^{-1})	P max. Net (x10^{-10} cel^{-1})	P max. Gross (x10^{-10} cel^{-1})	I k	R (x10^{-10} chl^{-1})	R (x10^{-10} cell^{-1})
0		6.30	0.79	0.016	0.040	24.762	N.A.	N.A.	N.A.	N.A.	N.A.	-0.38	-7.32
4	UVO	3.60	0.61	0.022	0.017	60.278	2.95	3.49	7.25	8.55	133	-0.55	-1.30
	UVT	5.50	0.42	0.007	0.079	12.727	2.58	4.02	5.62	8.32	57	-1.45	-2.70
	UVA	5.50	0.74	0.012	0.044	22.545	3.52	3.77	7.45	12.25	70	-0.25	-4.80
24	UVO	6.30	0.70	0.013	0.048	20.952	4.03	5.36	6.88	8.98	82	-1.33	-2.10
	UVT	1.40	0.14	0.020	0.007	142.857	N.A.	N.A.	0.60	8.20	N.A.	-1.58	-2.20
	UVA	3.50	0.58	0.029	0.012	82.857	2.77	3.59	6.30	8.00	120	-0.82	-1.70
48	UVO	1.90	0.42	0.025	0.008	130.000	1.45	1.79	5.20	6.85	150	-0.34	-1.10
	UVT	2.70	0.45	0.023	0.004	224.167	1.59	1.94	5.90	6.80	222	-0.40	-1.25
	UVA	2.70	0.41	0.025	0.005	205.833	2.67	3.47	6.45	8.15	125	-0.75	-1.70

Key to Table 2:

chl.	chlorophyll *a* (pg)
α	light utilization efficiency (μmole O_2 mg^{-1} chl. *a* (or $cell^{-1}$) μmole^{-1} quanta m^{-2})
a*	*in vivo*, spectral average, chl. *a* specific absorption cross section (m^2 mg^{-1} chl. *a*)
ϕ	maximal quantum utilization (mole O_2 μmole^{-1} quanta)
$P_{max.}$	maximum net or gross photosynthetic rate at saturating irradiance (μmole O_2 μmole^{-1} chl. *a* $min.^{-1}$, or μmole O_2 $cell^{-1}min.^{-1}$)
I_k	irradiance at onset of light saturation (μmole quanta m^{-2} s^{-1})
R	dark respiration (μmole O_2 μmole^{-1} chl. *a* $min.^{-1}$, or μmole O_2 $cell^{-1}min.^{-1}$)
N.A.	data not available

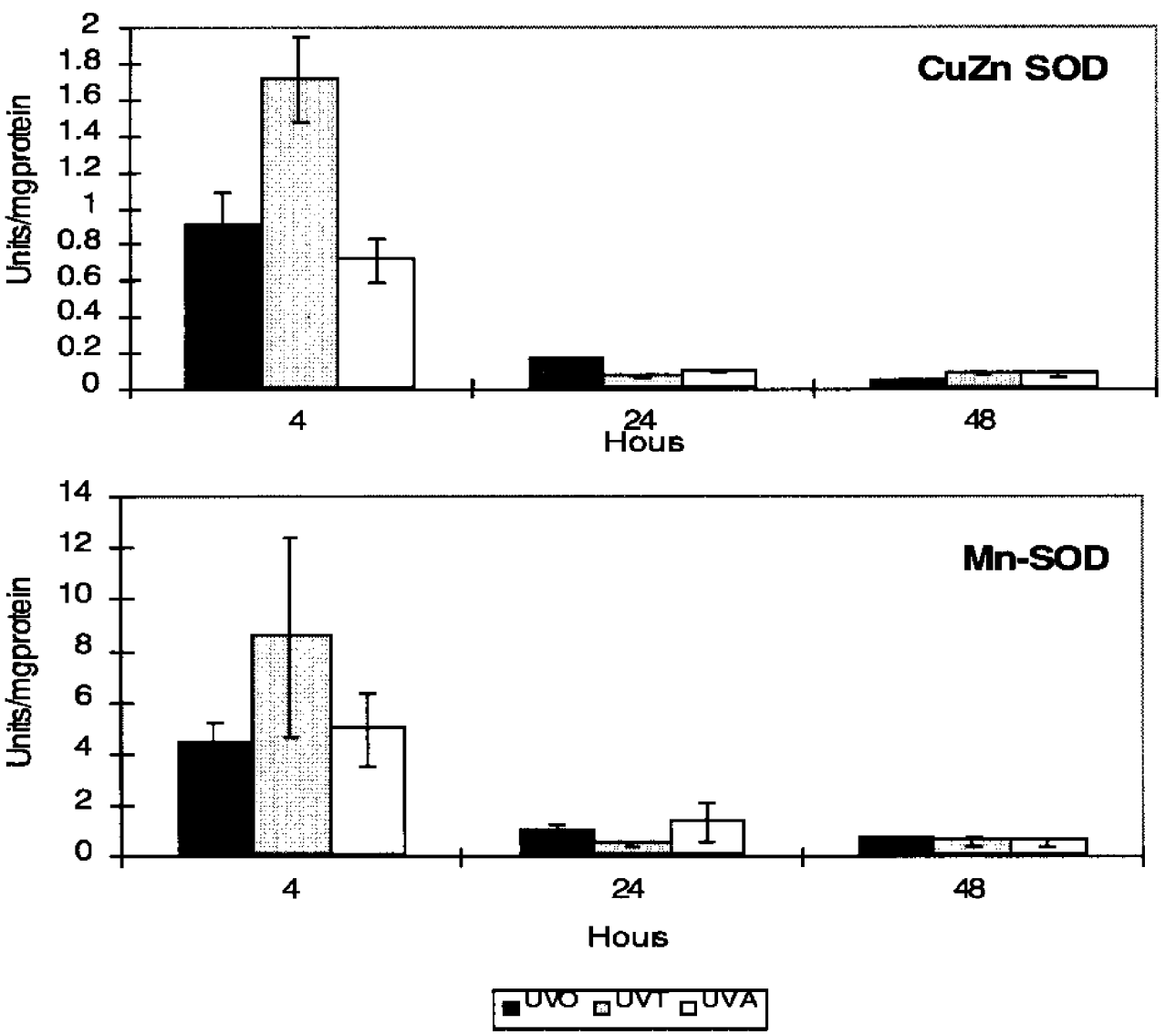

Figure 5. The effect of UV radiation on CuZn SOD and Mn SOD activity in *Nannochloropsis*. Units are absolute units of activity.

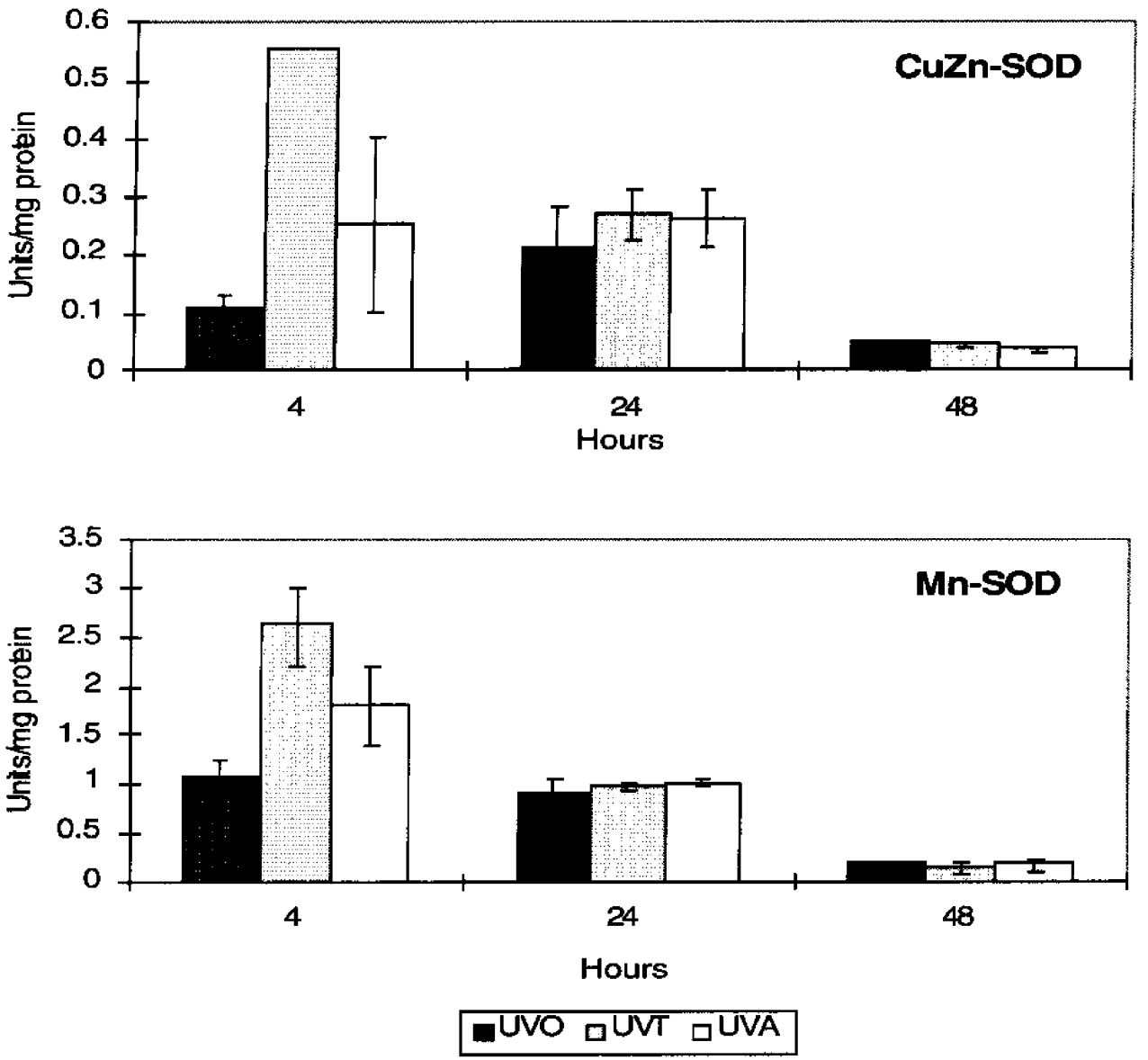

Figure 6. The effect of UV radiation on CuZn SOD and Mn SOD activity in *Dunaliella*. Units are absolute units of activity.

Antioxidative activity

SOD activity in both species showed a general decrease with time; different irradiation regimes apparently altered the dynamics of SOD activity for each time and for each species. Both *Nannochloropsis* and *Dunaliella* showed an increase in Mn SOD and CuZn SOD-specific activity after only 4-hours exposure to UVT (UV-A + UV-B + PAR) as compared to UVO (PAR only) or UVA (PAR + UV-A) (Figs. 5 and 6). There was a drop in *Dunaliella* CuZn SOD activity after 48-hours for all treatments, although no notable differences were observed between the treatments when measured after 24- or 48-hours. There was a significant drop in both *Nannochloropsis* CuZn SOD and Mn SOD activity after 24-hours for all treatments, althoughthe differences between treatments was slight. After 48-hours, the only discernible difference in activity was slightly higher CuZn SOD in UVT and UVA treated algae. Overall, SOD activity in

Nannochloropsis was greater than that of *Dunaliella*, and Mn SOD activity was higher than CuZn SOD for both algal species.

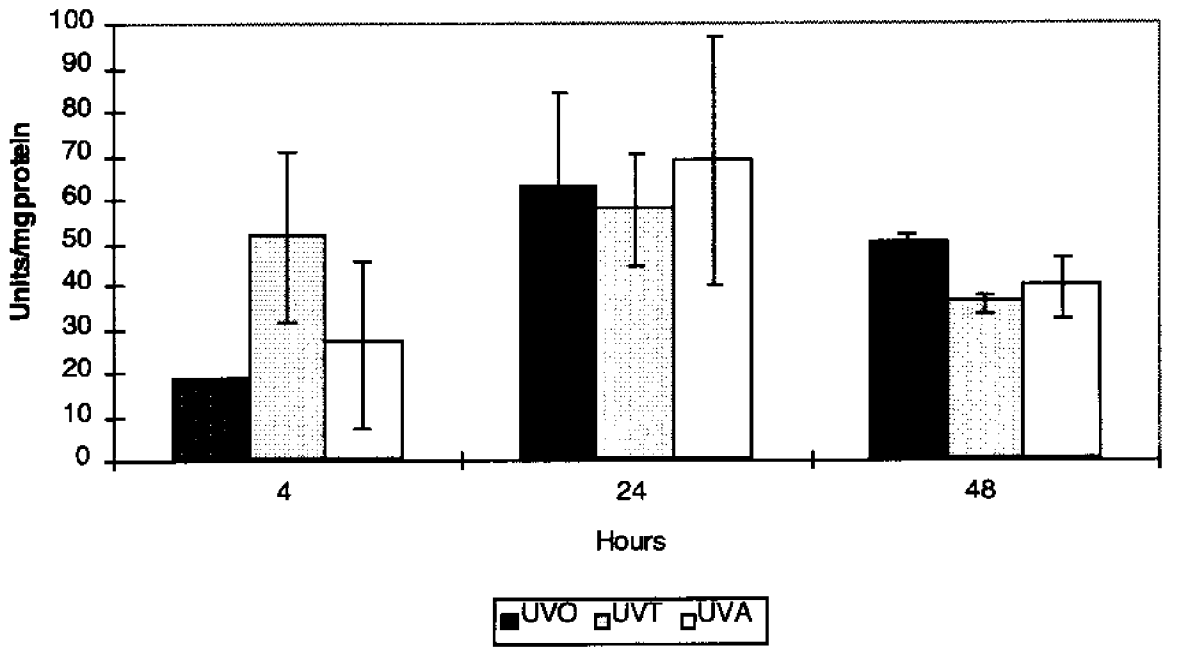

Figure 7. The effect of UV radiation on catalase activity in *Nannochloropsis*.

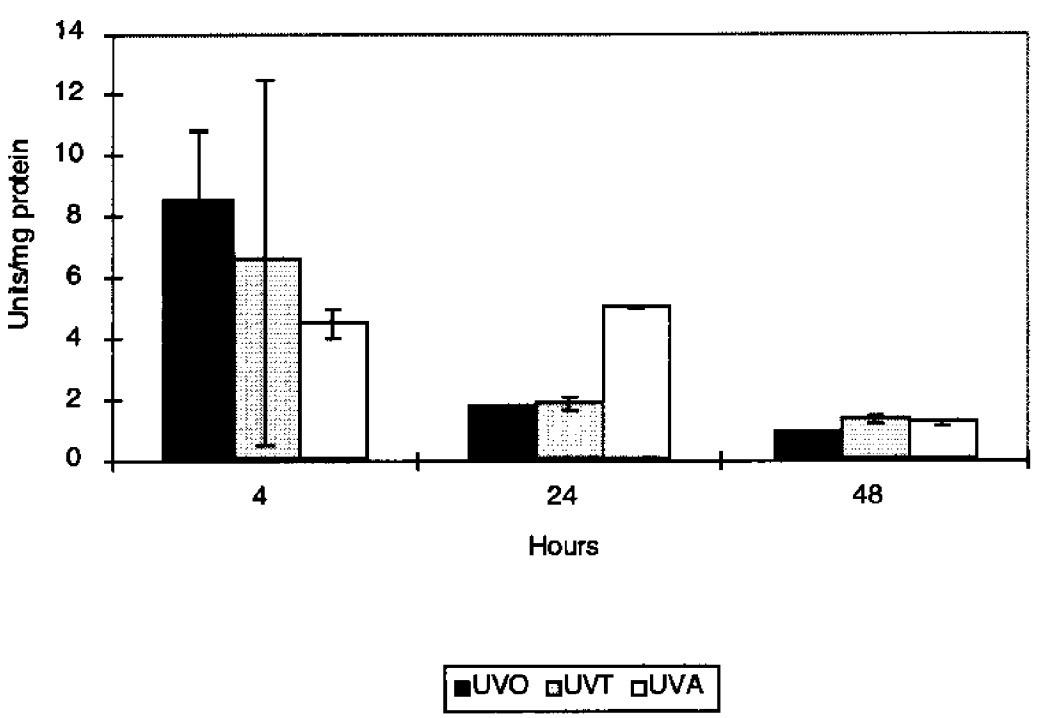

Figure 8. The effect of UV radiation on catalase activity in *Dunaliella*.

Nannochloropsis catalase activity in both the UVO and UVA treatment increased relative to the UVT treatment after 4-hours (Fig. 7). There was no significant difference between treatments after 24-hours; after 48-hours, there was a discernible difference in activity under UVO conditions when compared with UVT.

UV-A + PAR (UVA) caused a significant decrease in catalase activity relative to PAR-only (UVO) in *Dunaliella* after 4-hours (Fig. 8), but this changed after 24-hours to prompt a significant increase in activity (relative to UVO and UVT treatments). After 48-hours exposure, both UVT and UVA treatments gave higher catalase activities than UVO in *Dunaliella* .

Rubisco and LHCPII

Rubisco and LHCPII for *Nannochloropsis* were analyzed from stained PAGE gels (Fig. 9), and the results detailed in Table III. After exposure to UVA and UVT treatments for up to 24-hours, Rubisco levels in *Nannochloropsis* decreased, respectively, by factors of 1.6 and 2.0. In this alga, LHCPII levels also dropped after 4-hours; by a factor of 3.0 for UVA and 1.7 for UVT treatments. After 24- and 48-hours, there was no significant change in LHCPII levels. No clear trend was discerned in the effects of UV radiation on Rubisco and LHCPII levels in *Dunaliella*.

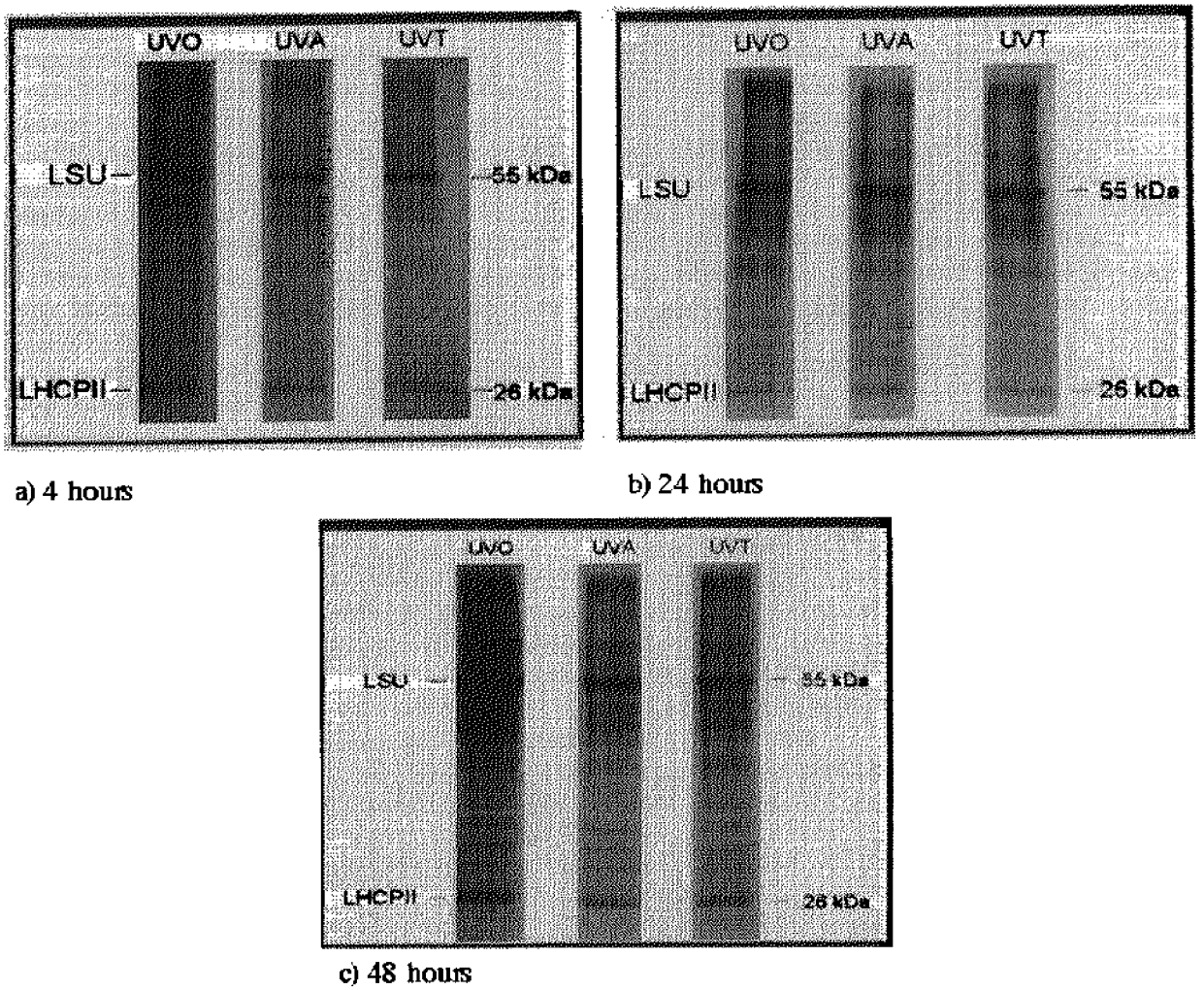

Figure 9. 12.5 % SDS-PAGE of *Nannochloropsis* extracts showing Rubisco and LHCPII proteins after a) 4h, b) 24h, and c) 48h exposure to UVO,UVT and UVA.

Table III. Effect of UV radiation on photosynthetic parameters for *Dunaliella*.

Time (hours)	Treatment	α (x 10^{-6} chl.$^{-1}$)	α (x 10^{-1} cell^{-1})	a *	ϕ	I/ϕ	Pmax. (x 10^{-6} chl.$^{-1}$) Net	Pmax. (x 10^{-6} chl.$^{-1}$) Gross	Pmax. (x 10^{-10} cell^{-1}) Net	Pmax. (x 10^{-10} cell^{-1}) Gross	I_k	R (x 10^{-6} chl.$^{-1}$)	R (x 10^{-10} cell^{-1})
4	UVD	6.63	7.33	0.093	0.079	13.641	5.59	8.85	104.00	142.00	117	-24.20	-38.00
	UVT	10.00	15.87	0.017	0.060	16.700	N.A.	N.A.	N.A.	N.A.	N.A.	-33.30	-79.00
	UVA	11.00	14.71	0.016	0.070	14.273	6.99	8.95	15.00	18.80	140	-19.60	-36.00
24	UVD	8.90	8.49	0.020	0.045	22.360	7.95	13.48	126.00	202.00	100	-55.30	-76.00
	UVT	5.53	6.70	0.024	0.023	43.750	5.21	9.80	75.50	102.50	239	-33.00	-52.50
	UVA	7.49	9.75	0.006	0.109	9.165	4.20	8.18	116.50	163.00	183	-29.90	-46.50
48	UVD	6.60	11.51	0.016	0.041	24.545	N.A.	N.A.	N.A.	N.A.	N.A.	-6.10	-14.00
	UVT	7.80	7.67	0.023	0.034	29.615	N.A.	N.A.	N.A.	N.A.	N.A.	-8.40	-12.00
	UVA	7.60	7.28	0.018	0.042	23.947	4.07	5.33	95.00	119.00	100	-12.60	-24.00

Key to Table 3:

chl. chlorophyll *a* (pg)

α light utilization efficiency (µmole O_2 mg^{-1} chl. *a* (or cell^{-1}) µmole^{-1} quanta m^{-2})

a* *in vivo*, spectral average, chl. *a* specific absorption cross section (m^2 mg^{-1} chl. *a*)

ϕ maximal quantum utilization (mole O_2 µmole^{-1} quanta)

$P_{max.}$ maximum net or gross photosynthetic rate at saturating irradiance (µmole O_2 µmole^{-1} chl. *a* min.$^{-1}$, or µmole O_2 cell^{-1}min.$^{-1}$)

I_k irradiance at onset of light saturation (µmole quanta m^{-2} s^{-1})

R dark respiration (µmole O_2 µmole^{-1} chl. *a* min.$^{-1}$, or µmole O_2 cell^{-1}min.$^{-1}$)

N.A. data not available

DISCUSSION

This set of experiments set out to discern the potential physiological and molecular damage caused by natural radiation to the photosynthetic machinery in two marine algae; *Nanochloropsis* and *Dunaliella*. The importance of antioxidant enzymes in the detoxification of intracellular free radicals formed by UV-A and UV-B was inferred from the results of these investigations. Despite a fluctuating radiation environment and limited time for research, it was shown that ultraviolet radiation adversely affected the ability of two diverse marine algae to fix carbon. UV-B radiation significantly inhibited *Nannochloropsis*' photosynthetic parameters, probably as a result of the inhibition of Rubisco which directly controls P_{max}. A similar trend was also shown in *Chaetoceros* (Hazzard, 1993). In both *Nannochloropsis* and *Dunaliella*, partial destruction of LHCPII was caused by UV-A and, to a much lesser extent, by UV-B radiation. It appears that there is a differential sensitivity to UV-A and UV-B by photosynthetically important proteins, as well as by different algal species. UV-A is also involved in photo-enhanced cell repair and actually assists in cell survival (Karentz *et al.*, 1991).

Both *Nannochloropsis* and *Dunaliella* have the potential to counter the increased risk posed by UV radiation. This was suggested by the rapid increase in SOD (Mn and CuZn) levels in both species and catalase in *Nannochloropsis* exposed to UV-A + UV-B + PAR (UVT). It is our conclusion that UV-B radiation alone caused an increase in O_2^- radicals and H_2O_2 which, in turn, induced higher activities of SOD and catalase production. During this early period of antioxidative activity, there was a simultaneous response in certain photosynthetic parameters indicating a weakening of the system due mainly to the UVT treatment. For example, after 4-hours exposure to UVT, *Nannochloropsis* showed decreases in radiation utilization efficiency, optical cross section, quantum utilization, and overall photosynthesis. The simultaneous increase in catalase and SOD activity, however, may have aided in the eventual recovery of photosynthesis. Additionally, in *Nannochloropsis*, there was an overall increase in carotenoids and in the ratio of carotenoids to chlorophyll *a*, which would also have afforded additional protection to the cells. It is possible these algae have UV-protective pigments (i.e., mycosporine-like amino acids) which could also aid in the photoadaptive process (Smith *et al.*, 1992). Another indicator that the cells were less stressed after 48-hours, possibly due to photoadaptation and repair of cell damage, was the decreased respiratory rate for all treatments.

Besides the obvious morphological differences between the two species that were chosen, it was shown that they also have characteristic antioxidative mechanisms. For example, *Nannochloropsis* (which is 40 times smaller than *Dunaliella*) had much higher SOD and catalase levels (at time 0), possibly because it is more readily swept to the water surface, and is less able to avoid UV radiation than the denser flagellate.

Alternatively, the higher protective activity may have been a local response to the initial switch from indoor growth at moderate irradiation to high outdoor irradiation intensities. The results also indicate that the turnover of Mn SOD and Cu/Zn SOD in *Nannochloropsis* is perhaps slower than that of *Dunaliella* since, after 24-hours exposure, SOD activity still fluctuated under the different irradiation treatments. It is also possible that the DNA repair mechanisms in *Nannochloropsis* may be more severely affected by UV-B present in the UVT treatment, hence the relative decrease in SOD after 24-hours. These conjectures require further in-depth research not only on a physiological level, but on a transcriptional level as well.

An overall comparison of the effects of UV radiation on *Nannochloropsis* and *Dunaliella* indicated that the larger alga, *Dunaliella*, showed superior photosynthetic ability and irradiation utilization efficiency, and was better adapted to long-term stress. Fewer significant physiological or biochemical changes were seen. It is suggested that the smaller *Nannochloropsis* is more sensitive, yet better adapted to sudden, short-term exposures to UV radiation stress. This was indicated by the relatively high turnover and reconstitution of Rubisco and LHCPII by *Nannochloropsis*.

Table IV: The effect of different uv regimes on the Rubisco and LHCP11 proteins (SDS PAGE analysis). Values expressed in % area of protein according to densitometer/10µg protein. The standard deviation for duplicate treatments varied +/- 5 - 10% of the average given values.

Nannochloropsis						
	Rubisco			**LHCP11**		
Time (h)	4	24	48	4	24	48
UVO	41	47	33	51	29	33
UVT	23	23	33	30	38	35
UVA	34	28	33	17	32	25
Dunaliella						
UVO	14	25	24	15	23	15
UVT	14	28	33	14	25	20
UVA	14	26	29	15	18	17

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Distribution of mycosporine-like amino acids in the tissues of Hawaiian Scleractinia: A depth profile

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ABSTRACT: The tissues of five species of Hawaiian hermatypic corals were found to contain concentrations of Mycosporine-like amino acids (MAAs) inversely correlated to depth and dose of UV radiation. Four of the six depth profiles conducted resulted in highly significant ($P<.01$) linear relationships between MAA concentration and UV radiation level. *Montipora verrucosa*, *Montipora patula*, *Porites compressa*, *Pocillopora damicornis* and *Pocillopora meandrina* were collected at a series of depths in and outside of Kane'ohe Bay, O'ahu, HI and analyzed using HPLC to identify and quantify the UV-absorbing compounds. Eight known and two unknown compounds with absorption maxima ranging from 313 to 360 nanometers were separated and quantified. Spectroradiometric measurements were made simultaneously during sampling to quantify and analyze the light regime at the collection sites.

INTRODUCTION

Hermatypic corals have evolved to flourish in environments characterized by clear, oligotrophic water. The transparency of this water results in an extremely low attenuation coefficient, and organisms living there are exposed to large fluxes of UV radiation (Jerlov, 1950). UV-A (wavelengths 320 - 400 nm) and UV-B (280 - 320 nm) radiation has been found to cause substantial physiological damage to organisms exposed at irradiance levels commonly emitted by the sun (Caulkins, 1982), especially at low latitudes where the ozone layer is thinner allowing higher levels to reach the Earth's surface. Targets of adverse effects in biological systems include the information carrying nucleic acids and proteins. The recognition of UV radiation as a selective pressure within a coral reef community has been well documented (Jokiel 1980). Behavioral adaptations such as avoidance, diurnal migration and cryptic lifestyles are not available to sessile organisms, specifically corals that need to be exposed to the sun for their photosynthetic zooxanthellae symbionts. This lifestyle presupposes the existence of a protective mechanism against the sun's damaging rays. First discovered in corals by Shibata (1969), a group of water soluble compounds (since termed Mycosporine-like Amino Acids or MAAs) absorbing in the 320 nm range have been found to perform this function. These compounds have been identified and quantified in many marine and terrestrial organisms (Chalker & Dunlap, 1990). Dunlap *et al.* (1986) conducted a study of the bathymetric distribution of MAAs in corals of Australia and found a considerable decline in the concentration of S-320 compounds in *Acropora* spp. with depth. This pattern was presumed to be an adaptation to the amount of UV radiation found at those depths. In this study, quantitative analysis of light regime has been correlated with concentration of seven described MAAs in five species of corals. Corals were collected along a depth gradient at two sites varying drastically in spectral quality, one inside of a barrier reef and one on an open-ocean island. A comprehensive baseline survey of the MAAs occurring in Hawaiian corals was also conducted in this study.

MATERIALS AND METHODS

Coral Collection

Corals were collected using snorkeling and SCUBA techniques on the windward side on O'ahu, Hawai'i (21° N, 157° W) during the summer months of June and July. Corals were collected at two sites; inside Kane'ohe Bay at a site called the Sliver Reef, and outside of the bay in open ocean water at Moku Manu Island. The conditions inside the bay are characterized by a high attenuation of light due to a high level of particulate matter. The open ocean site at Moku Manu displays relatively transparent, oligotrophic water with a low level of attenuation of light through the water column. The spectral irradiance (300-700 nm) was quantitatively measured at both sites using a LiCor LI-1800UW scanning spectroradiometer (LiCor, Lincoln, Nebraska). The cosine-corrected collector and sensors were programmed to scan from 300-700 nm in 2 nm intervals. All measurements of ambient solar irradiance were made at approximately 12 pm. For each depth

three scans were taken and the mean reported in units of mW m^{-2} nm^{-1}. The instrument was deployed and coral samples simultaneously collected using SCUBA. *Montipora verrucosa* was collected inside Kane'ohe Bay (Sliver Reef) at 1.5 m, 3 m, 4.6 m, 6.1 m, 7.6 m and 9.1 m, and at Moku Manu at 9.1 m, 12.2 m, 15.2 m, 18.3 m, and 21.3 m. *Pocillopora meandrina* and *Montipora patula* were collected at 6.1 m, 9.1 m, 12.2 m, 15.2 m, 18.3 m, and 21.3 m at Moku Manu. *Porites compressa* and *Pocillopora damicornis* were collected at 1.5 m, 3 m, 4.6 m, 6.1 m, 7.6 m and 9.1 m at Sliver Reef. Branches of each colony were harvested and placed in plastic bags for transport back to the lab where they were immediately frozen at -50°C until the time of extraction.

Analysis of Mycosporine-like amino acids by HPLC

Coral samples were thawed, cleaned of epiphytes, broken into small pieces, and placed in a known volume of 100% HPLC grade Methanol to extract overnight at -20°C. The extraction and analysis of MAAs were performed according to the procedures in Dunlap and Chalker (1986), Dunlap *et al.* (1986), and Shick *et al.* (1992). Samples were extracted in 5 cm^3 HPLC grade 100% methanol. Individual MAAs were separated by reverse-phase isocratic HPLC on a Brownlee RP-8 column protected with an RP-8 guard, in an aqueous mobile phase including 0.1% acetic acid and 45% methanol. Detection of peaks was by UV absorbance at 313 and 340 nm. Identities of peaks were confirmed by co-chromatography with standards of Mycosporine-glycine, shinorine, porphyra-334, palythine, asterina-330, palythinol, and palythene. Peaks were integrated and quantification of individual MAAs was accomplished using the quantitative standards listed and by on-line diode array spectroscopy. All MAAs were normalized to µmol MAA/mg protein using the soluble protein from an aliquot of the extracted sample. Protein was analyzed using the procedure described in Lowry *et al.* (1951).

Statistical analysis

Linear regression analysis was conducted to assess the possibility of an explanatory-response relationship between MAA concentration and UV radiation level. A linear relationship between the explanatory variable, UV radiation level, and the response variable, concentration of MAAs, was hypothesized. It was hypothesized to be linear because UV radiation (and light) has been shown to attenuate exponentially with depth, and the hypothesized notion that MAA concentration would also decrease exponentially with depth. Since these two variables are hypothesized to vary together in an exponential manner with depth, it follows that there would be a linear relationship between the two variables themselves.

RESULTS

The concentrations of MAAs in all five species of coral analyzed in this study clearly display an inverse relationship with depth. In all samples, there was a single MAA high in concentration relative to the others detected which showed this decreasing pattern most vividly. *Montipora verrucosa*, the species that was collected at both Kane'ohe Bay and Moku Manu, revealed the same pattern at both sites (Fig. 1). The MAA highest in concentration in this species was palythine, which showed a decrease of around two orders of magnitude from the shallowest to deepest collection. Concentrations of MAAs in *Pocillopora meandrina* (Fig. 2) and *Montipora patula* (Fig. 3) from Moku Manu were plotted both against depth (m) and UV dose (W m^{-2}). Concentrations of MAAs decreased with UV dose as they did with depth. Mycosporine glycine was found in high concentrations in *P. meandrina*, whereas in *M. patula*, palythine was the most abundant MAA. *Porites compressa* collected inside Kane'ohe Bay (Fig. 4) showed a two orders of magnitude decrease in asterina, but an unknown MAA showed an unusual pattern peaking in concentration at 5 m (approximately midway between surface and bottom).

The spectral data collected at the two sites (Figs. 5 & 6) show that radiation in the lower PAR wavelengths and the UV portion of the spectrum are attenuated at much shallower depths inside the bay than outside. The amount of integrated energy reaching the bottom of Kane'ohe Bay at 10 m was 0.59 W m^{-2}, and at Moku Manu the value at this depth was 15.8 W m^{-2}. At Moku Manu (21 m) there was 6.86 W m^{-2} of total UV reaching the bottom on the day of collection. The attenuation of light through a depth of 21 m at Moku Manu seems to be similar to the attenuation of light in only 10 m of water column inside Kane'ohe Bay.

The results of the linear regression analysis of the relationship between MAA concentration and UV radiation level (Table I) are highly significant for four of the six depth profiles: *M. patula*

(p<.001), *P. meandrina* (p=.003), *M. verrucosa* at the Kane'ohe Bay site (p=.005) and *P. compressa* (p=.005). R-squared values were also very high, signifying a strong causal relationship between the explanatory variable, UV dose, and the response variable, concentration of MAAs.

Table I. Linear regression analysis.

Depth profile	Site	ANOVA F	P value	Rsq
M. patula	MM	457.9	.000 *	99.1
P. meandrina	MM	42.4	.003 *	91.4
M. verrucosa	MM	6.22	.088	67.4
M. verrucosa	KB	31.95	.005 *	88.9
P. damicornis	KB	7.51	.052	65.2
P. compressa	KB	32.14	.005 *	88.9

*= P value of less than .01 significance level

DISCUSSION

The concentration of MAAs in the tissues of four species of corals have been found to be inversely related to the amount of UV radiation reaching the coral colonies. *Montipora verrucosa* sampled over a 23 m depth gradient outside the bay showed a similar two-orders of magnitude decrease in concentration of palythine as the sample collected inside the bay where the water is only 10 m deep. The spectral irradiance data, showing a similar level of attenuation of UV radiation at both sites even though the water column was twice as deep inside the bay, supports the speculation that the corals are responding to some sort of cue associated with light regime, and not simply depth. This study supports the idea that MAAs are indeed acting as UV radiation blockers in coral tissues because corals of the same species were found to contain very different concentrations of MAAs at similar depths when sampled from areas differing drastically in light regime.

The results of the linear regression analysis between MAA concentration and UV light level are very supportive of the hypothesis that this is indeed an explanatory-response relationship. This study supports the hypothesis that the corals are responding to the level of UV radiation, resulting in the accumulation of higher concentrations of MAAs in high UV environments. There is need for caution, however, because many parameters of the marine environment decrease with depth, and pinpointing which one is explicitly responsible will be quite difficult. It has been suggested that increased water motion and its association with increased metabolism may act as a cue to increase MAA production (Jokiel & Lesser, 1994). However, it still remains to be confirmed that the coral is harvesting MAAs from the symbiotic algae, and not either obtaining them through diet or producing MAAs themselves. Presently the only known origin of MAAs is the Shikimate pathway, a complex metabolic tree that produces the aromatic amino acids, plastoquinones, vitamins E and K, and many more compounds in photosynthetic microorganisms and higher plants (Bently, 1990). Considering the diversity of non-symbiotic organisms that contain MAAs, they must be obtaining these compounds through diet, just as most animals obtain the essential amino acids. A non-symbiotic coral, *Tubastraea coccinea*, does contain high levels of MAAs,

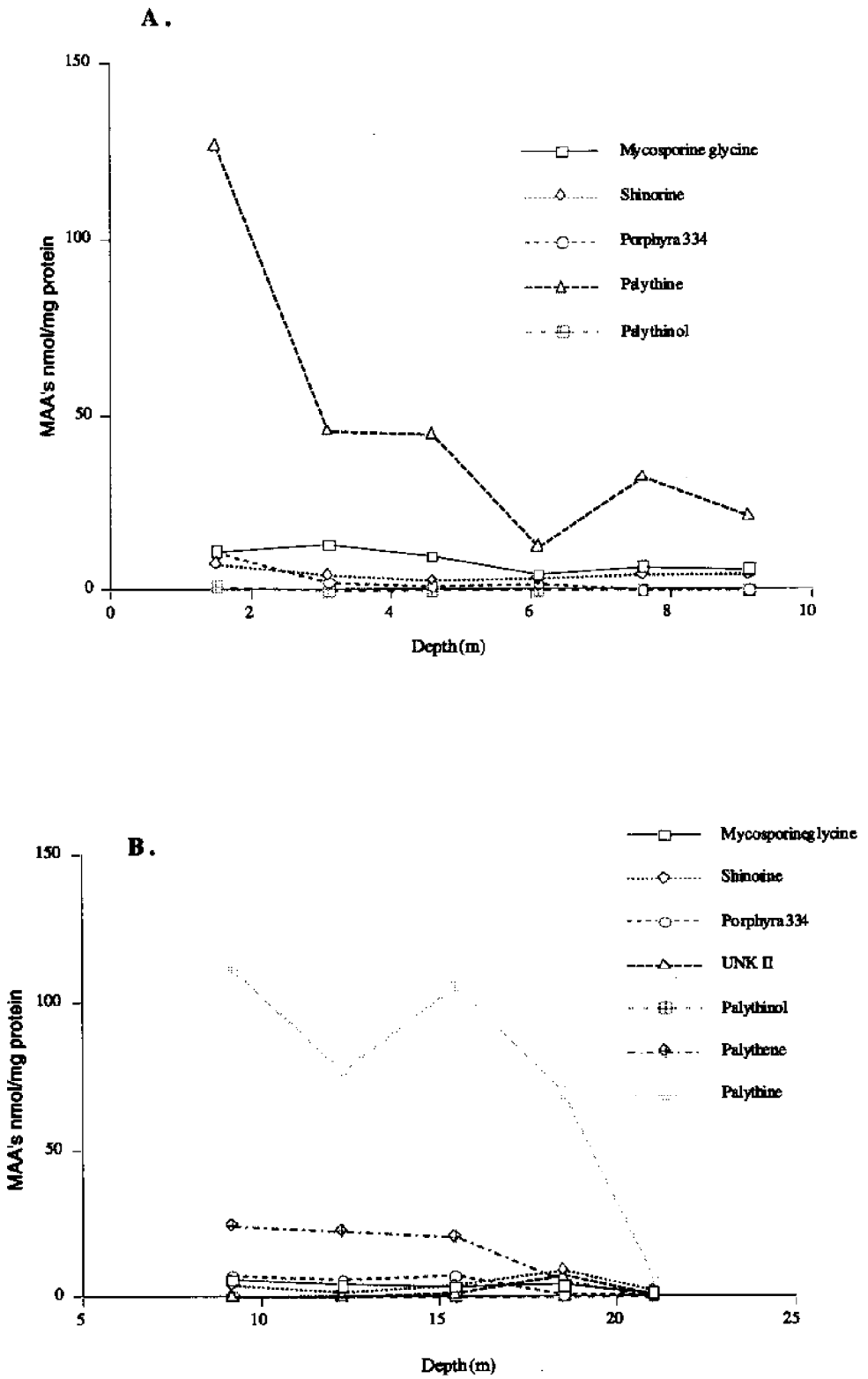
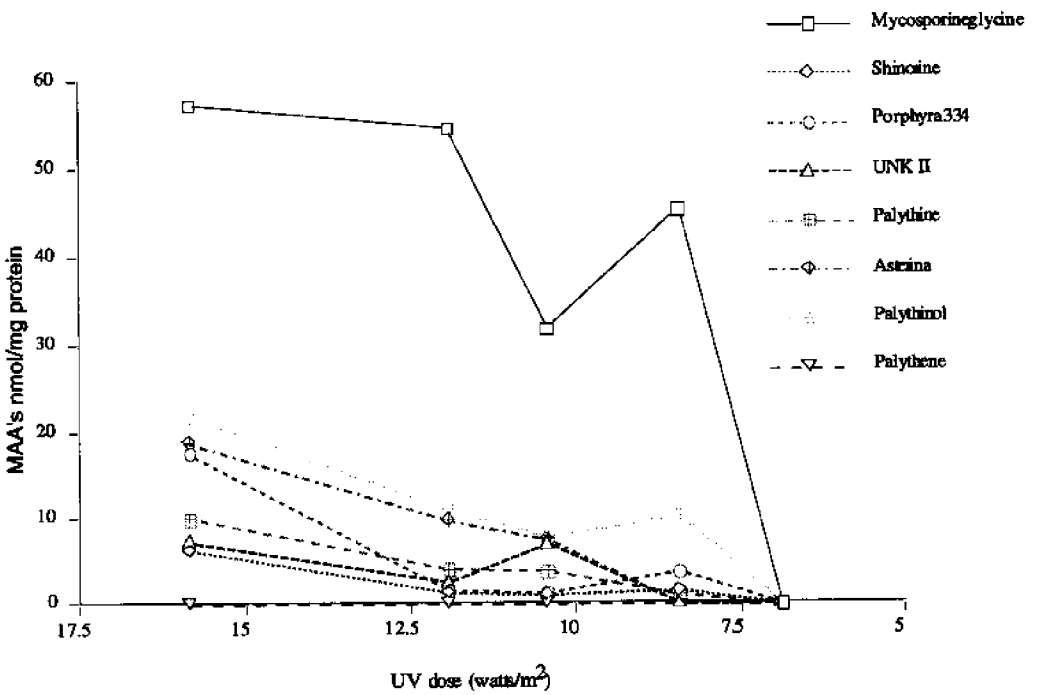


Figure 1. *Montipora verrucosa* MAA concentrations versus depth for Kane'ohe Bay (A) and Moku Manu (B).



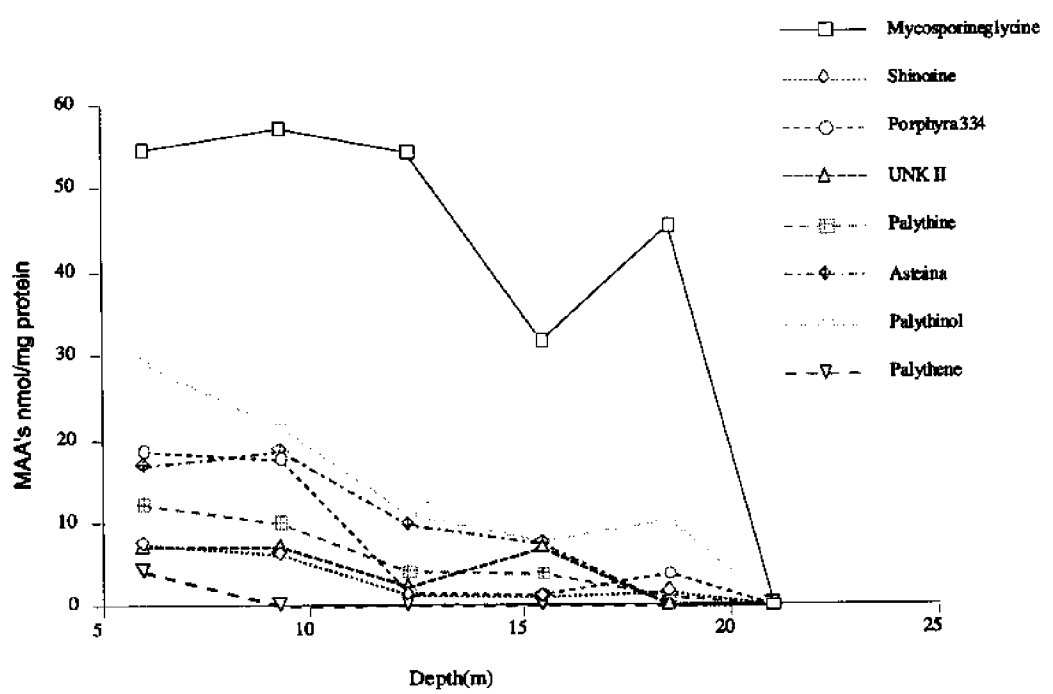


Figure 2. *Pocillopora meandrina* MAA concentrations from Moku Manu plotted both against depth (m) and UV dose (W m^{-2}).

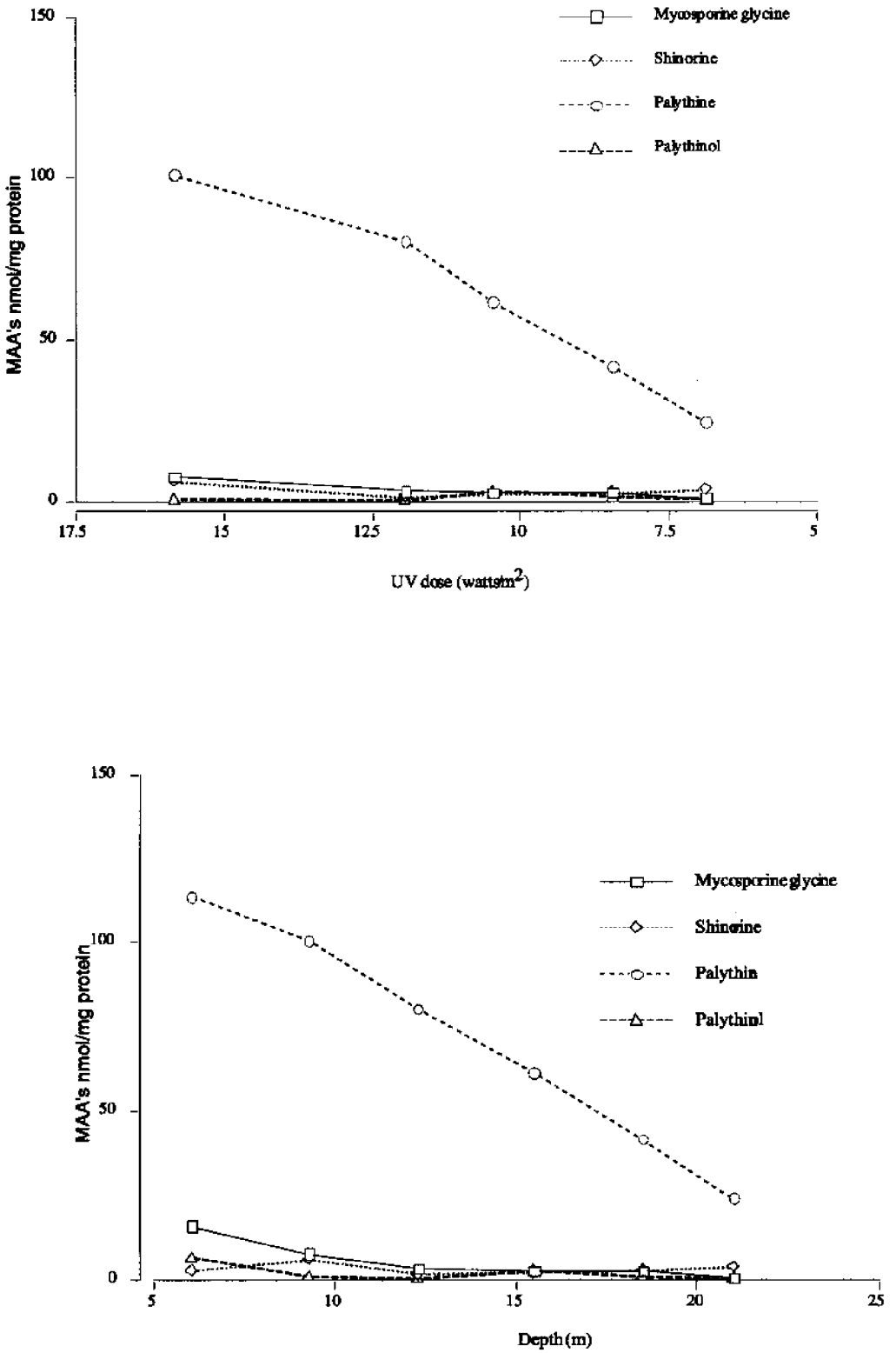


Figure 3. *Montipora patula* MAA concentrations from Moku Manu were plotted both against depth (m) and UV dose (W m^{-2}).

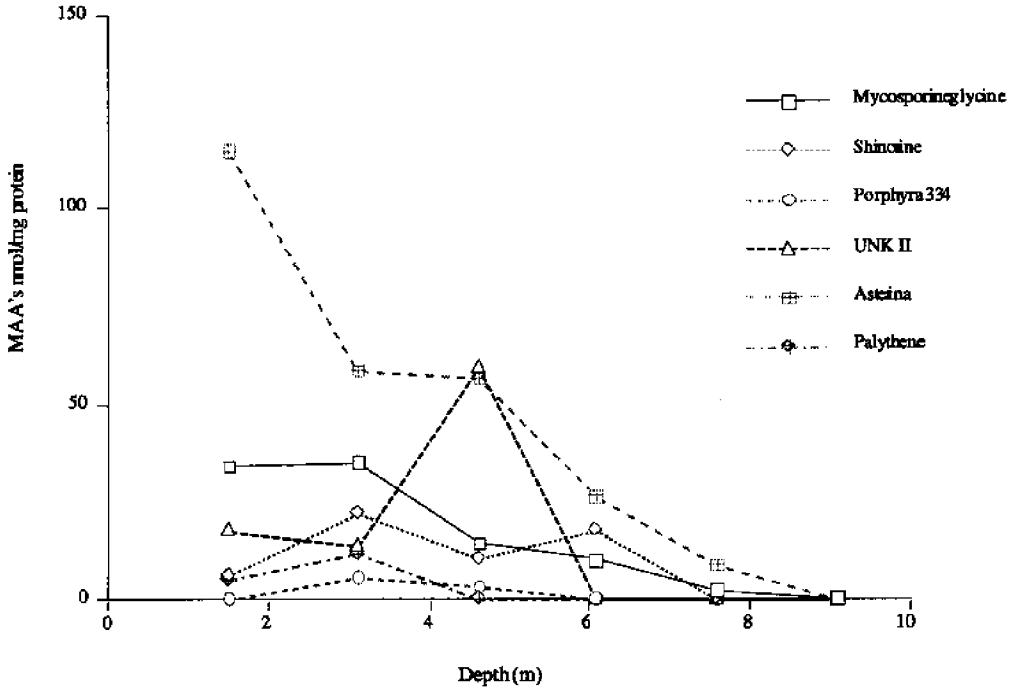


Figure 4. *Porites compressa* MAA concentrations versus depth in Kane'ohe Bay.

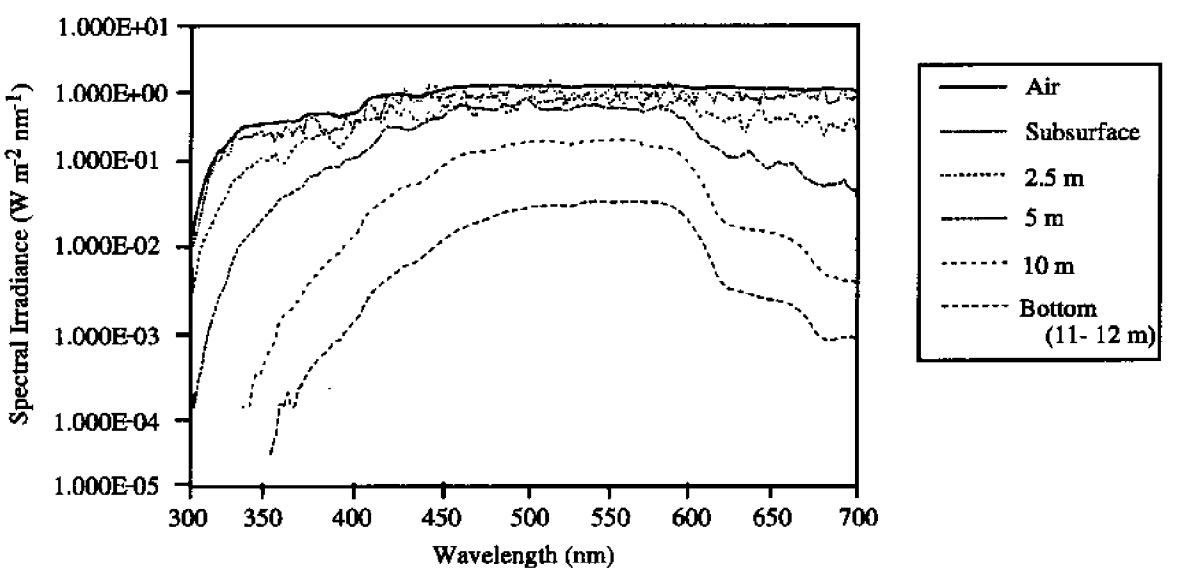


Figure 5. Spectral data collected at the Sliver Reef, Kane'ohe Bay.

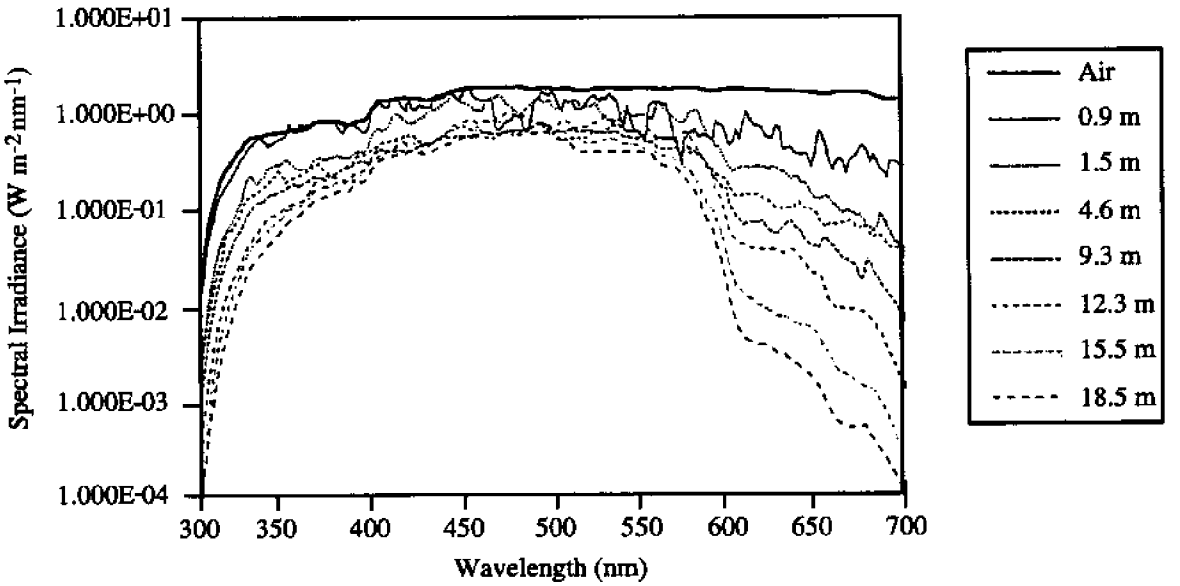


Figure 6. Spectral data collected at Moku Manu.

supporting the idea that corals can obtain MAAs through diet. The mechanism that is responsible for storing appropriate concentrations of MAAs in response to light regime is completely unknown. If corals harvest MAAs from their zooxanthellae, it is possible that increased concentrations of MAAs are a result of an increased rate of photosynthesis due to higher PAR and higher water motion (lower diffusion factor), and it is simply coincidental that high levels of MAAs are found in the tissues of corals that are found closest to the surface in high water motion, high UV environments.

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Abstract only

UV-absorbing compounds in the coral *Pocillopora damicornis*: interaction effects of light, water flow and UV radiation.

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ABSTRACT: A direct relationship exists between dose of solar ultraviolet (UV) radiation and concentration of mycosporine-like amino acids (MAAs) in the Hawaiian reef coral *Pocillopora damicornis*. However, MAA concentration is also influenced by flow regime and flux of photosynthetically active radiation (PAR). High fluxes of UV radiation in reef environments are correlated with high PAR and high water velocity, because all three parameters diminish exponentially with increased depth. This correlation is further strengthened along horizontal gradients on reefs. The clearest water is typically found on outer reefs growing in oceanic water. These ocean reefs typically experience high water velocities due to ocean swell when compared to the more turbid lagoon reefs exposed only to small wind-driven waves. Flow-modulated photosynthetic rate appears to be a major factor effecting the observed changes in MAA concentration when this coral is grown under different flow regimes and identical fluence rates of UV and PAR radiation. High PAR and/or high water velocity significantly enhance the effect of increased UV radiation on MAA concentration. Thus, observed differences and changes in MAAs under different environmental conditions might not directly reflect differences or changes in UV radiation flux unless all other parameters are equivalent.

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Response of a Pacific stony coral to short-term exposure of ultraviolet and visible light

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ABSTRACT: Colonies of the Pacific stony coral *Montipora verrucosa* were transplanted from 10m depth to an *in-situ* respirometer at 0.5 m depth. The corals were exposed for 1 to 2 days to full sun or 30% sun, without UV-A or UV-B, with UV-A but not UV-B, or with both UV-A and UV-B. Metabolic measurements were taken continuously for each coral and levels of chlorophyll and MAA were determined at the culmination of the experiment. No significant interaction between ultraviolet (UV) radiation effects and visible irradiance (photosynthetically active radiation = PAR) effects was observed. Corals exposed to full sun showed significantly lower maximum net photosynthesis rates, respiration rates, and photosynthetic efficiency, but net P:R ratios, compensation point, and saturation point were unchanged. These results suggest that increased visible irradiance was detrimental to both the photosynthetic algae and to the coral tissue. Maximum net photosynthesis rates and chlorophyll *a* levels were lower in corals exposed to UV, but respiration rates remained the same. This may indicate that UV was damaging to the photosynthetic algae but not the coral tissue. There was no significant difference between effects of UV-A and effects of UV-A + UV-B for any response variable. These results are important because they indicate that corals respond dramatically even to very short-term exposure to both increased visible irradiance and to increased UV irradiance.

INTRODUCTION

Tropical coral reefs are regularly exposed to high levels of visible irradiance, or photosynthetically active radiation (PAR, 400 nm - 700 nm), and ultraviolet radiation (UV-A, 320 nm- 400 nm and UV-B, 280 nm - 320 nm) (Cullen & Neale, 1993; Gleason, 1993). While it has long been recognized that high visible irradiance reaches these reef organisms, historically it was believed that UV radiation was not a significant influence. Although high levels of UV reach low latitude ocean surfaces due to the thinness of the ozone layer and the low zenith angle of the sun (Baker *et al.*, 1980), it was believed that these short wavelengths were attenuated rapidly and efficiently by the water and, therefore, did not reach reef organisms (Smith & Baker, 1979). However, it is now well known that UV radiation penetrates to considerable depth in tropical oceans (Jerlov, 1950; Jerlov, 1968; Smith & Baker, 1979; Fleischmann, 1989).

Concern is mounting over the potential increase in UV radiation reaching coral reefs as ozone depletion continues (Hader and Worrest, 1991). Reef organisms may not be able to adapt quickly enough to survive the changing conditions. On shorter time scales, episodic events such as unusually calm periods may result in dramatic water column clearing as witnessed at bleaching locations in the Caribbean in 1987 and 1990 (Goenaga *et al.*, 1988; Gleason & Wellington, 1993). These water column clearing events can provide for greater exposure of reef organisms to both UV radiation and visible irradiance.

Reef organisms can employ three main defense mechanisms against UV radiation: avoidance, protection, and repair. The sessile nature of stony corals coupled with the dependence of the coral-zooxanthellae symbiosis on solar radiation necessitates that corals be exposed to UV radiation. Therefore, corals are left with two options: protect themselves, and be capable of repair should damage occur.

In shallow water marine environments, it is believed that many sessile invertebrates employ UV absorbing compounds to protect themselves from the damaging effects of UV radiation. These compounds, formerly known as "S-320" (Shibata, 1969), are collectively known as mycosporine-like amino acids (MAAs) with absorption maxima in the 310 - 360 nm range (Hirata *et al.*, 1979; Tsujino *et al.*, 1980; Karentz *et al.*, 1991). It has been suggested that hermatypic corals synthesize or accumulate their own suites of MAAs as protection against this radiation (Dunlap & Chalker, 1986; Dunlap *et al.*, 1986). Jokiel and York (1982) observed a decrease in these compounds when UV was blocked from *Pocillopora damicornis*, and Maragos (1972) observed decreased concentrations as depth increased.

UV radiation has been implicated in damaging organisms both in terrestrial and aquatic systems (Harm, 1980; Wood, 1987; Cullen & Neale, 1993). Worrest *et al.* (1981a; b) correlated altered species compositions and standing crops of algae with increased long-term UV dosage. Lesser & Shick (1989) reported 30% lower growth rates in zooxanthellae from *Aiptasia pallida* acclimated under high visible light conditions with UV radiation than those acclimated under high visible light conditions without UV or acclimated in low light conditions. Jokiel and York (1982; 1984) also found reduced growth rates in a number of algal species, including zooxanthellae, when exposed to visible light with UV-A + UV-B radiation. There is also evidence of UV induced

photosynthetic inhibition (Sisson, 1986; Lesser & Shick, 1989). Studies of this photoinhibition suggest UV damages or destroys chlorophyll and/or chloroplasts (Gessner & Diehl, 1951; Smith *et al.*, 1980; Hader & Worrest, 1991). Lesser and Shick (1989) found reduced levels of chlorophyll in *Aiptasia pallida* in the presence of ultraviolet radiation. It has been suggested that increased UV radiation has been instrumental in causing widespread bleaching observed in tropical oceans (Fisk & Done, 1985; Harriott, 1985; Oliver; 1985; Goenaga *et al.*, 1988). However, the evidence is strictly correlational and is confounded by increases in visible irradiance.

The shorter wavelength, higher energy UV-B radiation, is considered more biologically damaging than UV-A (Cullen & Neale, 1993). Bothwell *et al.* (1994) discovered that UV-B disrupts many photosynthetic processes including pigment stability, electron transport system, and photosystem II reaction centers. Despite the belief that UV-B is more damaging than UV-A (Calkins & Thordardottir, 1980), numerous studies of UV effects have not investigated these components independently (but, see Jokiel & York, 1984). It is important to consider that while UV-B photons may be more damaging per photon than UV-A, there are much greater fluxes of UV-A in the ocean than UV-B. Bothwell *et al.*(1994) concluded that although UV-B is more disruptive, higher photon flux in UV-A usually produces the majority of inhibition of photosynthesis in algae.

There is some debate regarding the relative contribution of visible irradiance and UV radiation in damaging reef organisms. Brown *et al.* (1994) speculate that the bleaching patterns observed in corals in Thailand result from longer wavelength, photosynthetically active radiation (PAR) and that UV radiation played a nominal role, if any at all. They contend that there is increasing evidence that high levels of PAR negatively affect algal photosynthetic systems (review in Powles, 1984). Contrastingly, Jokiel and York (1984) consider that the role of PAR in photoinhibition has been overestimated and that long-term photoinhibition effects are primarily caused by UV radiation. They discovered that algae in their study could rapidly photoadapt to increased PAR (92% surface irradiance), but the addition of UV resulted in growth photoinhibition. Their study agreed with previous studies (Steemann-Nielsen, 1962; Steemann-Nielsen *et al.*, 1962; Prezelin & Matlick, 1980) that showed that some microalgae can rapidly photoadapt to high levels of visible light (<< 24 hrs). This debate can only be settled by further, non-correlational research on the relative impacts of these two light components. In addition to the impact of UV radiation or visible irradiance on reef organisms, there may be an interaction between these two effects. It is possible that the combination of these two factors produces greater detrimental effects than either of the two acting alone. It is equally possible that one factor may ameliorate the effects of the other.

The experiments in this study were designed to test for acute effects of short-term exposure of the Hawaiian stony coral, *Montipora verrucosa* to ecologically realistic levels of increased visible irradiance, increased UV radiation (both UV-A and UV-B), and the interaction of the two. The specific questions addressed were: (1) Does the metabolic ability of the Hawaiian stony coral, *Montipora verrucosa*, change with increased visible irradiance and/or UV-A and/or UV-B radiation? (2). Does chlorophyll content change with increased visible irradiance and/or UV-A and/or UV-B radiation? (3). Do MAA levels change with increased irradiance and/or UV-A and/or UV-B radiation?

MATERIALS AND METHODS

Collection Site and Study Organism

This study was conducted at the Hawai'i Institute of Marine Biology (HIMB), Coconut Island, Kane'ohe, Oahu during the summer of 1994. All coral pieces were collected off the Coconut Island's Lighthouse Dock from large colonies of plating *Montipora verrucosa* at a depth of 10 m. A pair of coral pieces were taken from the same location on each colony; one piece of the pair was used in the experimental treatments while the other piece of the pair was used immediately for lab analysis to obtain initial estimates for chlorophyll *a* levels, and MAA levels. A total of 36 pieces from 18 colonies was used for this experiment.

Experimental Design

The collected pieces of *M. verrucosa* were transported approximately 300 m in shaded fresh seawater to the site of the in-situ respirometer in the evening before each experimental run. The

respirometer was located on a suspended platform at a constant depth of 0.5 m. Six pieces of *M. verrucosa* were collected at a time. One piece from each pair was randomly placed in each of the three chambers of the respirometer, while the other pair-member was taken to the lab for immediate processing.

The treatments were established in a 2(PAR) x 3(UV) x 2(Days) incomplete factorial design. The three UV treatments were established by placing filters over each respirometer chamber. One filter only allowed PAR to pass, one filter allowed PAR+UV-A to pass, and the third filter allowed PAR + UV-A + UV-B to pass. Two visible irradiance treatments were crossed with this set of three UV treatments. Neutral density screening was used to create two PAR levels: full light intensity and simulated 10 m light intensity (30% surface intensity at the coral collection site). The factorial design is incomplete in that a second day of treatment was applied only to the corals exposed to the full light PAR treatment. All combinations of treatments were replicated 3 times.

Metabolic Measurements

Photosynthetic measurements were made using the suspended respirometer. The experimental corals were placed inside sealed 2.3 liter plexiglass chambers for the duration of the experiment. The chamber lids were quartz and therefore transparent to UV-A and UV-B radiation. Each chamber was connected to a submersed impeller pump which fully exchanged all the water in the chambers every hour. Temperature readings taken periodically inside the chambers showed that this flushing rate prevented significant heating inside the chambers (< 0.5 °C higher than surrounding water). Uniformity of oxygen levels throughout the chamber was achieved by rotating stir bars below a perforated pedestal that held the coral. Oxygen production (photosynthesis) and consumption (respiration) were measured by YSI oxygen probes and recorded every 4 minutes by an Omnidata datalogger. Light was measured by a LiCor light meter and 4pi steradian spherical sensor attached to the respirometer and recorded every 4 minutes.

All the oxygen data was downloaded from the datalogger to a computer immediately after each run. The recorded voltage readings were converted into oxygen (ppm) and light (uE m^{-2} s^{-1}) units. Rates of oxygen consumption and production were calculated and plotted against the irradiance values to develop light saturation curves. The curves were fit to the data using the following model:

$$P = R + (P_{max} - R)(1 - e^{-\alpha I})$$

This model yielded the following response variables: P_{max}, R, α, I_c, and I_k. P_{max} is the maximum net photosynthesis rate achieved by the coral. It is measured as the horizontal asymptote of the light saturation curve. R is the nighttime respiration rate for the coral. The initial slope (α) at the compensation irradiance (I_c) of the light saturation curve is termed the photosynthetic efficiency. It is the irradiance level at which the coral produces enough oxygen to compensate for its respiration and there is a net production of oxygen. I_k is the saturation irradiance. It is the irradiance at which the coral reaches its maximum net photosynthesis (P_{max}).

Three metabolic response variables (I_c, I_k, net P:R) were independent of normalizations. Three metabolic response variables (R, net P_{max}, alpha) were normalized in three ways: per cm^2 surface area, per gram wet weight, and per microgram chlorophyll *a*.

Surface Area Determination

After removal from the chambers, each coral was taken to the lab in seawater and surface area was determined. Each coral was videotaped and projected surface areas were then calculated. This value was then multiplied by 2 to determine the surface area of the coral involved in photosynthesis and respiration because *M. verrucosa* has tissue on both the top and underside of the plate.

Photosynthetic Pigment Determination

One pair-member was analyzed for chlorophyll immediately after collection from the field. As this piece was taken from an area of the colony immediately adjacent to the experimental coral piece, its chlorophyll level served as an estimate of the pre-treatment chlorophyll level of the pair member.

Chlorophyll levels were determined photometrically. A small plug was taken from the middle of each piece using a 1 cm diameter cork borer and then ground in 90% acetone. The ground

coral and solvent were placed in a dark refrigerator to extract overnight. The tubes were then spun in a refrigerated centrifuge at 3500 x g and the absorbance of the supernatant was then measured on a scanning spectrophotometer at 750 nm, 663 nm, and 630 nm. Chlorophyll *a* values were determined using the equations of Jeffrey and Humphrey (1975).

MAA Assays

MAA levels were determined using HPLC. A small plug was taken from the center of each coral with a 1 cm diameter cork borer and then placed in a -50°C freezer. When the experiment was completed, all frozen samples were sent to Dr. Michael Lesser (University of New Hampshire) where the samples were then extracted using methanol. The extract was separated using an HPLC and the peaks were quantified and identified using standards. All the MAAs were normalized by protein. Protein values were determined using the Lowery method (Lowrey, 1951).

Methods of Statistical Analysis

The response of an individual coral piece to a treatment effect may be influenced by pretreatment factors. Hence, a covariate analysis with these factors (ANCOVA) might provide a more powerful test than a simple non-covariate parametric test (ANOVA). Covariates were only used when the covariate model was significantly different than the reduced, non-covariate model. The choice between different significant covariate models follows the method outlined by C. L. Mallows (1973) (also see the description under PROC REG in SAS/STAT User's Guide, Volume 2, 1990). Independence of the covariates was tested by a linear correlation procedure (Dilorio, 1991).

Comparisons of treatment means were tested using t-tests, but only if the treatment effect first tested significant under an F-test. The significance level for all tests was 5%. All statistical analyses were carried out using a PC SAS package.

RESULTS

Covariate Analysis

The set of potential covariates were: MAA, chlorophyll, respiration normalized by surface area, respiration normalized by chlorophyll, and respiration normalized by wet weight. Each of these covariates represents a "before treatment" factor. The respiration rates of each experimental coral were measured during the night prior to exposure to the UV and PAR treatments. The chlorophyll and MAA estimates for each experimental coral were obtained from the bioassay of the corresponding pair member.

Chlorophyll and MAA were evaluated as covariates for all metabolic response variables. The respiration rates were only used for the metabolic response variables with the same normalization. Table I shows the correlation analysis for the covariates. The only covariates that showed a significant correlation were R by wet weight and R by surface area (correlation coefficient = 0.73, p=0.001). Since these two covariates were always used separately in any analysis, all covariate models tested used a set of independent covariates. Tables II, III, and V show the covariates used in the analyses. Of all the covariates tested, only two were used: Chl-before and MAA-before. An ANCOVA procedure was used to analyze the following response variables: R normalized by Chl using Chl-before as covariate, net P_{max} normalized by Chl using MAA-before as covariate, and net P_{max} normalized by surface area using MAA-before and Chl-before as covariates. All other response variables were analyzed using an ANOVA procedure.

Assay Analysis - Chl and MAA

Within 10 m light corals - UV effects:

Corals under shaded conditions were run for 1 day. For the MAA analysis, the before treatment chlorophyll level was a significant covariate, but there was no significant UV effect (p=0.58). For the chlorophyll *a* analysis, no covariate tested significant, and there was no significant UV effect (p=0.61).

Within full light corals - UV effects:

Corals exposed to full visible light were run for 2 days. No covariates tested significant in either the MAA analysis or the chlorophyll *a* analysis. There was no significant UV effect for total MAA levels (p=0.16) or chlorophyll *a* level (p=0.08).

The covariates used and the significance levels of the treatment effects are summarized in Table VII, and the means and standard errors are provided in Table VIII.

Metabolic Analysis Within Day 1 - UV and PAR Effects

There was no observed interaction between the UV treatment effects and the PAR treatment effects for any of the metabolic response variables. Significance levels for both unnormalized and normalized response variables are provided in Tables II and III, respectively. The means and standard errors are provided in Table IV.

UV Effects:

Compensation irradiance (I_c), saturation irradiance (I_k), and net P:R ratio did not show a significant UV effect (p=0.59, 0.39, and 0.52, respectively). There was no significant differences between the means for each of these response variables among the different UV treatments (Table IV).

Of the metabolic response variables normalized by surface area, wet weight, and chlorophyll, the only significant UV effect occurred with net P_{max} normalized to surface area (p=0.02, Table III). The corals that were shielded from UV had a higher net P_{max} than those exposed to UV-A (Table IV, Figure 1). However, this design could not detect a significant difference between those exposed to UV-A + UV-B from those shielded from UV or those receiving UV-A-only (means and t-groupings - Table IV).

The light saturation curves shown in Figure 2 provide an overall view of the UV treatment effects on the metabolism of *M. verrucosa*. The higher net P_{max} for corals receiving only visible light is evident.

PAR Effects:

There was no significant PAR effect on I_c, I_k, and P:R (p=0.57, 0.12, and 0.46, respectively, Table II, means - Table IV). There was a highly significant PAR effect for each of the metabolic response variables for each of the three normalizations. The 10m light corals showed significantly higher net P_{max}, R and alpha values (all $p < 0.05$ - Table III, means and t-groupings - Table IV). Figure 1 shows mean values and 95% confidence intervals of net P_{max} normalized by surface area. The same trend was evident for the other two metabolic response variables normalized by surface area, as well as for all metabolic response variables normalized by wet weight and chlorophyll.

Figure 3 shows the light saturation curves for the 2 PAR treatments, irrespective of UV treatment. They clearly indicate the effects of increased visible irradiance on the photosynthetic ability of *M. verrucosa*.

Metabolic Analysis Within Full Light Treatment - UV and Day Effects

There was no interaction observed between the UV treatments and the day of exposure for any of the metabolic response variables (Table V).

UV Effects:

When full light corals from the three UV treatments were compared for the first and second day of exposure, saturation irradiance (I_k) and net P:R ratio were not different among the UV treatments (p=0.72, and 0.33, respectively, Table V). However, there was a significant UV effect for compensation irradiance (p=0.01, Table V). Corals shielded from UV had significantly lower compensation points than those exposed to UV-A (means and t-groupings, Table VI). However, it was not possible to distinguish the compensation point of corals exposed to UV-A + UV-B from that of corals exposed to UV-A or shielded from UV (Table VI). Figure 4 shows the lower compensation irradiance for corals shielded from UV. Maximum net photosynthesis, respiration, and photosynthetic efficiency normalized to surface area, wet weight, and chlorophyll did not show significant UV effects (p values -Table V, means and standard errors - Table VI). Figure 5 shows the light saturation curves for the full light corals for both days of exposure to the different UV treatments. Although corals shielded from UV appear to have a higher photosynthesis, this was not significant.

Day Effects:

There was no significant difference for saturation irradiance or net P:R ratio between the first day and second day of exposure for the full light corals (p=0.14, and 0.70, respectively, Table V). However, the compensation irradiance was significantly lower during the first day of exposure and increased during the second day of exposure (p=0.002, Table V, means and t-groupings - Table VI, Figure 4).

Of the three variables normalized to surface area, wet weight, and chlorophyll, only respiration normalized to surface area showed a significant day effect (p=0.02, Table V, means and t-groupings, Table VI). Figure 6 shows that respiration rates were significantly higher on the second day of exposure.

DISCUSSION

Assay Analysis - Chl and MAA:

UV Effects:

The lack of a UV effect for chlorophyll or total MAA levels for full light or 10 m light corals should be considered in the context that the exposure time was only two days and one day, respectively. Further studies using larger sample sizes may determine whether or not chlorophyll *a* levels and total MAA levels change during short-term exposures to increased UV irradiance.

Previous studies have found that corals shielded from UV for an extended time tend to lose their MAAs, while corals exposed to higher levels of UV for an extended time tend to increase their MAAs (Jokiel & York, 1982; Scelfo, 1985). Kinzie (1993) found that M. verrucosa acclimated in PAR + UV had higher levels of these compounds than those acclimated in PAR only. Although these changes occurred after multiple weeks of exposure, it is not yet known how quickly corals of this species will change MAA levels. This experiment did not uncover any changes in MAA levels in 2 days.

Metabolic Analysis Within Day 1 - UV and PAR Effects:

The observation that no interaction occurred between UV radiation and visible irradiance after one day of exposure suggests that the detrimental effects of either treatment were not exacerbated or ameliorated by the other treatment. A previous study with freshly isolated zooxanthellae from the zoanthid, *Palythoa caribaeorum* have indicated that there can be a synergistic effect between these two factors (Lesser *et al.*, 1990).

UV Effects:

In this experiment, only one of the metabolic response variables, the maximum net photosynthesis rate, showed a significant UV effect after one day of exposure. The observation that net P_{max} was highest in those corals shielded from UV suggests that UV radiation may be damaging the photosynthetic components of zooxanthellae. These results are consistent with previous studies. For example, Kinzie (1993) found enhanced

photosynthetic ability in full sun by *Montipora verrucosa* acclimated to PAR + UV compared to corals acclimated to PAR only. Lesser and Shick (1989) found UV exposure decreased net P_{max} in freshly isolated zooxanthellae but not cultured zooxanthellae from *Aiptasia pallida*.

The inability to detect a difference in net P_{max} between corals receiving only visible light from those exposed to UV-A + UV-B allows 2 interpretations. First, increased levels of UV-B may ameliorate the effects of increased UV-A. Second, the experimental design was not sufficient to detect the difference. The first interpretation seems unlikely, and perhaps a follow-up study with an increased sample size would be able to make a distinction.

The lack of a UV treatment effect on the respiration rates indicates that UV is not affecting the coral tissue and is consistent with results obtained by Kinzie (1993). One day of exposure to increased UV did not significantly change the irradiance necessary for the corals to reach compensation (I_c) or to achieve saturation (I_k). It is important to consider that the UV effects observed occurred after very short-term exposures to naturally occurring levels of UV radiation.

PAR Effects:

Powles (1984) provides a review of evidence that high levels of PAR affect algal photosynthetic systems, causing photoinhibition and subsequently photoxidation at elevated doses over prolonged time. In this experiment, similar detrimental effects of increased PAR were observed after only 1-2 days of exposure. Net P_{max}, respiration rates and photosynthetic efficiency were all significantly lower in corals exposed to full visible irradiance. These results suggest that significant increases in PAR (perhaps due to water column clearing events) may interrupt the proper functioning of both the host coral and the zooxanthellae.

These results contrast with previous work by Jokiel and York (1984), who found remarkably high tolerances to PAR in the dinoflagellate, *Symbiodinium microadriaticum* (a symbiotic coral zooxanthellae). This alga demonstrated growth photoinhibition to increased levels of UV but, even at full surface intensity, visible irradiance produced no inhibitory effects.

Metabolic Analysis Within Full Light Treatment - UV and Day Effects:

The corals exposed to full visible irradiance were run for a second day to allow comparison of changes from the first day of exposure to the second day of exposure for the different UV treatments.

UV Effects:

Since corals exposed to UV-A had higher compensation irradiance than those shielded from UV, it suggests that UV-A is stressful to corals. However, the low sample size of the experiment did not allow any distinction to be determined between UV-A effects and UV-B effects or between PAR only and UV-A + UV-B.

Day Effects:

I speculate that the higher compensation irradiance and higher respiration rates observed during the second day of exposure are due to cumulative stress from the high levels of visible irradiance.

CONCLUSION

Exposing colonies of *Montipora verrucosa* that were photoadapted to light levels at a 10 m depth, to dramatically increased visible irradiance, appeared to detrimentally impact both the photosynthetic zooxanthellae as well as the coral tissue. These colonies exhibited decreased maximum net photosynthesis rates, respiration rates, and photosynthetic efficiency. Colonies exposed to dramatically increased UV irradiance did show a metabolic response, but did not respond to the same degree as to the increased visible irradiance. The response to the increased UV appeared to be limited to the symbiotic algae. Corals shielded from UV had higher maximum net photosynthesis rates but no other differences in metabolic response variables were

observed. Significantly, the treatment effects observed in this experiment occurred following exposure to natural levels for only 1 to 2 days.

Table I. Pearson correlation coefficients for analysis for covariates (* indicates significantly correlated, $p < 0.05$).

	MAA-before	Chl-before	R by SA	R by Wgt	R by Chl
MAA-before	1.00				
Chl-before	0.23	1.00			
R by SA	0.08	-0.23	1.00		
R by Wgt	-0.23	-0.27	0.73 (p=0.001 *)	1.00	
R by Chl	-0.007	-0.003	0.26	0.14	1.00

Table II. Metabolic data - comparison of first day of exposure for all treatments. Significance levels from ANOVAs for UV and visible light treatment effects for normalization independent variables (* indicates $p < 0.05$).

Response Var.	Covariates	UV Effect	PAR Effect	UVxPAR
I_c	none	0.59	0.57	0.06
I_k	none	0.39	0.12	0.99
P:R	none	0.52	0.46	0.62

Table III. Metabolic data - comparison of first day of exposure for all treatments. Significance levels from ANOVAs or ANCOVAs for UV and visible light treatment effects for variables normalized to surface area (SA), wet weight (Wgt), and chlorophyll (Chl) (* indicates $p < 0.05$).

Response Var.	Covariates	UV Effect	PAR Effect	UVxPAR
R by SA	none	0.88	0.0004 *	0.34
R by Wgt	none	0.91	0.003 *	0.62
R by Chl	Chl	0.29	0.003 *	0.73
P_{max} by SA	MAA+Chl	0.02 *	0.0002 *	0.84
P_{max} by Wgt	none	0.71	0.002 *	0.96
P_{max} by Chl	MAA	0.23	0.0001 *	0.13
Alpha by SA	none	0.86	0.001 *	0.74
Alpha by Wgt	none	0.98	0.004 *	0.91
Alpha by Chl	none	0.70	0.01 *	0.22

Table IV. Metabolic data - comparison of first day of exposure for all treatments. Mean values for metabolic response variables, sample sizes, and standard errors (letters represent t-test groupings. Values in different groups are significantly different at $p < 0.05$).

Treatment	Variable: I_c			Variable: I_k			Variable: P:R		
	N	Mean	SE	N	Mean	SE	N	Mean	SE
PAR-only	6	65.08	4.56	6	424.83	46.03	6	5.17	0.88
PAR+UV-A	6	72.02	6.16	6	351.33	32.55	6	3.62	0.67
PAR+UV-A+ UV-B	6	66.40	6.04	6	361.00	37.00	6	4.42	1.10
10m Light	9	66.19	3.42	9	413.33	18.63	9	4.99	1.00
Full Light	9	69.48	5.44	9	344.78	38.98	9	4.82	0.26

RESPIRATION		normalized by SA		normalized by Wgt		normalized by Chl	
Treatment	N	Mean	SE	Mean	SE	Mean	SE
PAR-only	6	-4.79	0.77	-10.20	1.58	-0.387	0.06
PAR+UV-A	6	-4.78	0.40	-10.99	1.54	-0.529	0.06
PAR+UV-A+ UV-B	6	-4.52	0.75	-10.50	1.90	-0.563	0.06
10m Light	9	-5.86 a	0.36	-13.25 a	1.13	-0.628 a	0.05
Full Light	9	-3.53 b	0.30	-7.88 b	0.69	-0.358 b	0.05

PMAX		normalized by SA		normalized by Wgt		normalized by Chl	
Treatment	N	Mean	SE	Mean	SE	Mean	SE
PAR-only	6	24.7 x	2.15	50.76	8.69	2.2	0.24
PAR+UV-A	6	16.4 y	2.11	41.12	11.43	1.7	0.24
PAR+UV-A+ UV-B	6	19.1 xy	2.06	45.13	12.95	2.3	0.24
10m Light	9	27.4 a	1.73	64.55 a	7.13	2.9 a	0.20
Full Light	9	12.7 b	1.73	26.79 b	4.43	1.2 b	0.20

ALPHA		normalized by SA		normalized by Wgt		normalized by Chl	
Treatment	N	Mean	SE	Mean	SE	Mean	SE
PAR-only	6	0.065	0.007	0.140	0.018	0.006	0.0004
PAR+UV-A	6	0.061	0.010	0.141	0.029	0.006	0.0012
PAR+UV-A+ UV-B	6	0.061	0.012	0.144	0.035	0.007	0.0016
10m Light	9	0.081 a	0.005	0.187 a	0.022	0.008 a	0.0010
Full Light	9	0.044 b	0.003	0.097 b	0.057	0.004 b	0.0007

Table V. Metabolic data - comparison of first day versus second day of UV exposure by full light corals. Significance levels from ANOVAs for metabolic response variables for UV treatment effect and day effect (* indicates $p < 0.05$).

Response Var.	Covariates	UV Effect	Day Effect	UVxDay Effect
I_c	none	0.013 *	0.002 *	0.781
I_k	none	0.723	0.137	0.991
P:R	none	0.332	0.703	0.921
R by SA	none	0.111	0.022 *	0.891
R by Wgt	none	0.263	0.087	0.991
R by Chl	none	0.721	0.515	0.959
P_{max} by SA	none	0.314	0.377	0.989
P_{max} by Wgt	none	0.346	0.385	0.901
P_{max} by Chl	none	0.333	0.39	0.907
Alpha by SA	none	0.074	0.302	0.948
Alpha by Wgt	none	0.203	0.372	0.494
Alpha by Chl	none	0.418	0.585	0.999

Table VI. Metabolic data - comparison of first day of exposure and second day of exposure by shaded corals. Mean values, sample sizes, standard errors for metabolic response variables (letters represent t-test groupings. Values in different groups are significantly different at $p < 0.05$).

	Variable: I_c			Variable: I_k			Variable: P:R		
Treatment	N	Mean	SE	N	Mean	SE	N	Mean	SE
PAR-only	6	69.32 x	5.25	6	442.00	66.54	6	4.97	0.98
PAR+UV-A	6	97.98 y	8.14	6	390.33	53.07	6	2.57	0.55
PAR+UV-A+ UV-B	6	81.75 xy	9.89	6	373.17	62.80	6	3.68	1.30
10m Light	9	69.48 a	5.44	9	344.78	38.98	9	3.99	1.00
Full Light	9	96.56 b	6.24	9	458.89	50.42	9	3.49	0.67

RESPIRATIO N		normalized by SA		normalized by Wgt		normalized by Chl	
Treatment	N	Mean	SE	Mean	SE	Mean	SE
PAR-only	6	-3.76	0.33	-8.23	0.93	-0.409	0.056
PAR+UV-A	6	-4.45	0.24	-10.21	1.20	-0.475	0.072
PAR+UV-A+ UV-B	6	-3.62	0.35	-8.22	0.69	-0.390	0.080
10m Light	9	-3.53 a	0.30	-7.88	0.69	-0.395	0.071
Full Light	9	-4.36 b	0.14	-9.89	0.80	-0.454	0.065

PMAX		normalized by SA		normalized by Wgt		normalized by Chl	
Treatment	N	Mean	SE	Mean	SE	Mean	SE
PAR-only	6	17.42	2.33	38.92	6.43	1.93	0.30
PAR+UV-A	6	11.29	2.40	25.16	5.17	1.14	0.23
PAR+UV-A+ UV-B	6	11.85	3.46	27.10	7.51	1.26	0.50
10m Light	9	11.93	1.74	26.79	4.43	1.24	0.21
Full Light	9	15.11	2.81	33.99	6.19	1.64	0.37

ALPHA		normalized by SA		normalized by Wgt		normalized by Chl	
Treatment	N	Mean	SE	Mean	SE	Mean	SE
PAR-only	6	0.048	0.0024	0.105	0.0053	0.005	0.0004
PAR+UV-A	6	0.038	0.0029	0.086	0.0086	0.004	0.0004
PAR+UV-A+ UV-B	6	0.037	0.0037	0.086	0.0086	0.004	0.0008
10m Light	9	0.043	0.003	0.097	0.0053	0.004	0.0004
Full Light	9	0.039	0.003	0.088	0.0080	0.004	0.0008

Table VII. Significance levels from ANOVAs and ANCOVAs for UV treatment effects for chlorophyll *a* (ug cm^{-2}) and total MAA (nmol mg^{-1} protein) for 10 m light corals after 1 day exposure and for full light corals after 2 days exposure.

	10m Light Corals - 1 day exposure		Full Light corals - 2 day exposure	
Response Var.	Covariates	UV Effect	Covariates	UV Effect
Chl *a*	none	0.61	none	0.08
Total MAA	Chl-before	0.58	none	0.16

Table VIII. Mean and standard errors for chlorophyll *a* (ug cm^{-2}) content and total mycosporine-like amino acids (nmol/mg protein) for 10m light corals and full light corals.

	10m LIGHT CORALS					FULL LIGHT CORALS				
		Chl *a*		Total MAA			Chl *a*		Total MAA	
UV Treatment	N	Mean	SE	Mean	SE	N	Mean	SE	Mean	SE
PAR-only	3	12.3	0.92	9.47	3.44	3	14.68	1.51	702.16	645.8
PAR+UV-A	3	7.53	0.92	49.48	14.32	3	13.07	1.65	175.72	120.0
PAR+UV-A+ UV-B	3	7.91	1.85	35.28	16.53	3	13.01	0.04	191.09	152.5

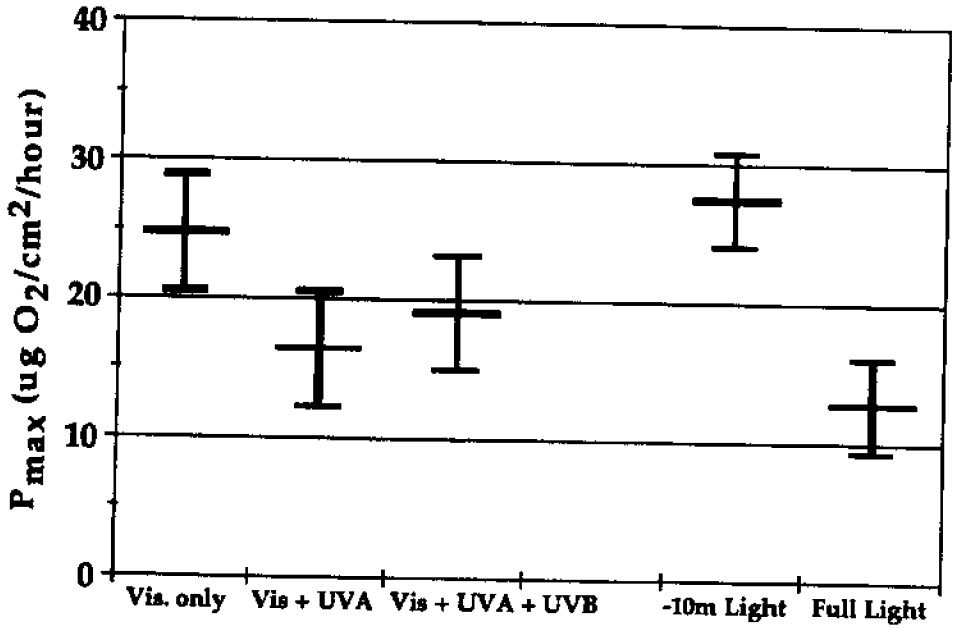

Figure 1. Significant UV and PAR treatment effects after one day of exposure for maximum net photosynthesis normalized by surface area. Means and 95% confidence levels.

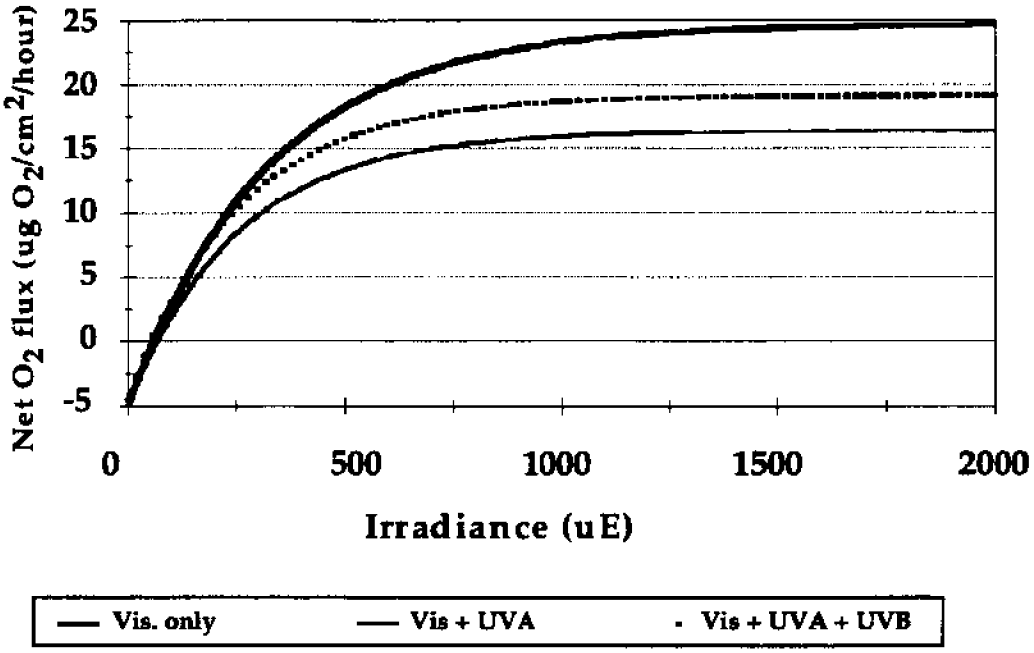

Figure 2. Light saturation curves for all corals for first day of exposure to the three UV treatments.

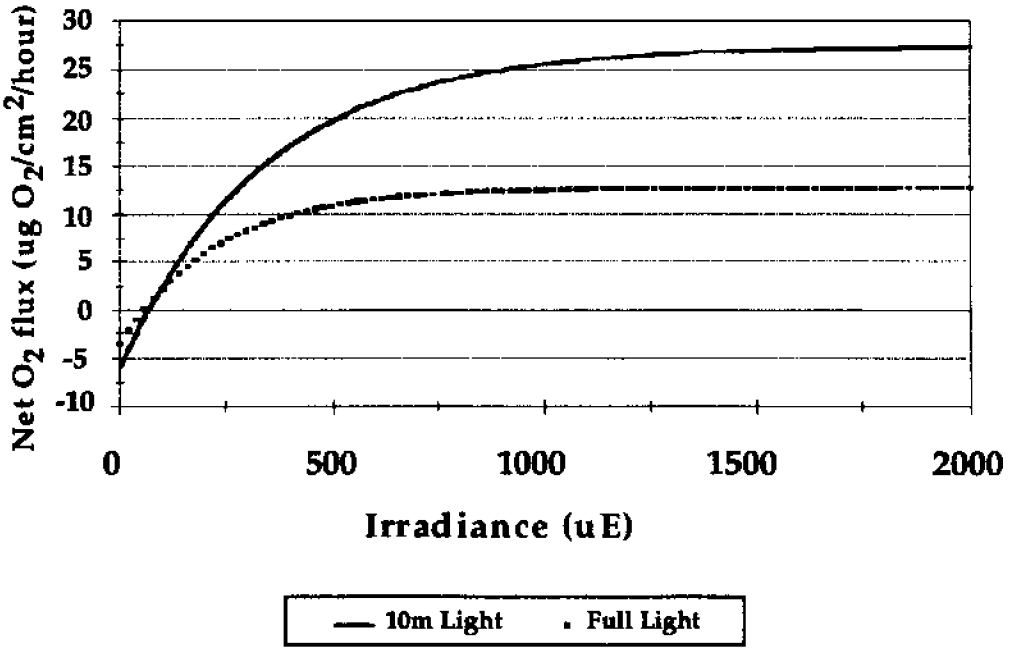

Figure 3. Light saturation curves for first day of exposure to the two different PAR treatments.

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Bleaching and lipids in the Pacific coral *Montipora verrucosa*

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ABSTRACT: Endosymbiotically-derived fixed carbon is drastically reduced in bleached corals as a result of decreased chlorophyll *a* per zooxanthella or the number of zooxanthellae per cm^2 of coral tissue. Under such conditions, corals may have to rely on other sources of energy including stored lipids. In this study, I investigated the relationship between coral bleaching and lipid concentrations when bleaching was induced by increased total solar irradiance.

I hypothesized that bleached corals would have a lower lipid level than non- bleached corals and that progressively longer bleaching periods would result in successively lower lipid concentrations. Bleaching was induced via increased solar irradiance by transplanting fragments of *Montipora verrucosa* from 10 m to 1 m depth for 4, 8 or 14 days. Corals appeared paler in color after 3 days of exposure and chlorophyll *a* concentrations were significantly lower in all bleached corals after 4, 8 and 14 days of exposure. Chlorophyll *a* levels in bleached corals did not recover to normal levels after 10 or 6 days following exposure. Lipid levels in bleached coral fragments did not differ significantly from control fragments at any time during the experiment. These data indicate that *M. verrucosa* does not depend on lipid energy reserves during short bleaching periods. Decreased metabolism, increased heterotrophy, gamete resorption or some combination of these factors during the early stages of bleaching may compensate for the immediate decrease in photosynthetically- derived fixed carbon in this species. Future studies on the changes in lipids over a longer period of time is needed in order to fully assess the importance of lipid reserves in bleached corals.

INTRODUCTION

Over the past 15 years the incidence of widespread bleaching events has increased on coral reefs throughout the world. Coral bleaching is characterized by the loss of photosynthetic pigments and has been correlated to factors such as elevated seawater temperatures, increased ultraviolet radiation or combinations of the two (Coles & Jokiel, 1978; Jokiel, 1980; Hoegh-Gulberg & Smith, 1989; Cook *et al.*, 1990; Glynn, 1993; Gleason & Wellington, 1993). Bleaching can result in interruption of coral growth, reduction of reproductive output and eventually death (Jokiel & Coles, 1977; Glynn & D'Croz, 1990; Szmant & Gassman, 1990). The magnitude of bleaching events and the rate of coral recovery varies within and between both sites and species (Brown & Suharasono, 1990; Fitt *et al.*, 1993). The reasons for these variations in bleaching responses however are poorly understood. One physiological component that may account for inter- and intra-specific differences in bleaching susceptibility is lipid concentrations. Under standard physiological conditions, fatty acids and glycerol are synthesized by zooxanthellae from photosynthetically–fixed carbon and are translocated to the host where they are either metabolized or transformed and stored primarily in the form of wax esters and triglycerides (Batey & Patton, 1984). Lipids in corals are stored predominantly in the animal host with concentrations ranging from 29% of dry biomass in *Montastrea annularis*, to 30- 40% of dry biomass in various Hawaiian species (Harland *et al.*, 1992; Stimson, 1987). However, under bleached conditions corals may rely heavily on lipid stores due to decreases in the number of zooxanthellae and/or chlorophyll per zooxanthella which leads to lower photosynthetic rates, and thus reduced quantities of translocated lipid. In the Caribbean, temperature- stressed corals showed 39- 73% lower lipid concentrations than non-bleached conspecifics 6 months after the onset of the event (Porter *et al.*, 1989). This lower lipid level was presumed to be the result of an estimated 50% decrease in translocated carbon. However, it is not known whether or not lipid levels decrease in corals bleached by increased solar irradiance nor how rapidly lipid levels change in response to bleaching. In this study I examined the initial effects bleaching by solar irradiation had on the total lipid content of the Pacific coral, *Montipora verrucosa*. I hypothesized that bleached corals would have a lower lipid level than non-bleached corals, and that progressively longer bleaching periods would result in successively lower lipid concentrations. Also, given that increased solar irradiance causes chlorophyll levels to decrease, I expected lipid and chlorophyll *a* levels in corals to be correlated (Hoegh–Gulberg & Smith, 1989; Gleason & Wellington, 1993). Finally, I briefly monitored lipid and chlorophyll *a* levels in the corals following exposure to increased irradiance, since little is known about coral recovery following short bleaching periods.

MATERIALS AND METHODS

The experiment was conducted between July 12 and July 26, 1994, at the Lighthouse Point (LP) and Bridge to Nowhere (BTN) sites on Coconut Island, Hawai'i. Fifteen coral colonies of the plating morph of *Montipora verrucosa* were tagged at a depth of 10 m at the LP site. Two fragments with minimum dimensions of 15 cm^2 were broken off from each of these parent colonies. One fragment was transplanted to a depth of 1 m at the BTN site (treatment fragment) and the other fragment remained directly beside the parent colony as a control for transplantation (control fragment)(Fig. 1). Transplanted and control fragments were affixed to a uniform substrate plastic mesh platform. Treatment fragments were induced to bleach by exposing them to increased solar irradiance via transplantation from a low light (LP site at 10 m) to a high light site (BTN at 1 m) for 4, 8 or 14 days. Transplanted coral fragments were exposed to a 70% increase in average total irradiance over that observed at 10m (based on integrated light measurements made every 2 nm using a LiCor Li-1800UW underwater spectroradiometer between 300 - 700 nm). Integrated UVB (300 - 320 nm), UVA (320 - 400 nm) and photosynthetically active radiation (PAR 400 - 700 nm) levels were approximately 99%, 93% and 66% greater at 1 m than at 10 m respectively. Treatment fragments were transplanted to the BTN site to better replicate the low wave action conditions present at 10 m at the LP site (LP site is beside a dock with high boat traffic). These sites are separated by approximately 100 m, occur in the same small lagoon and have similar sedimentation and temperature regimes. Hourly temperature values were recorded both at the 1 m BTN and at the 10 m LP site using two HOBO brand miniature data loggers. The two sites differed by less than 1.0°C on average and neither temperature regime was high enough to induce bleaching (LP average temp. = 26.5 ± 2.0 °C and BTN average temp. = 27.0 ± 1.0°C)(Jokiel & Coles, 1977).

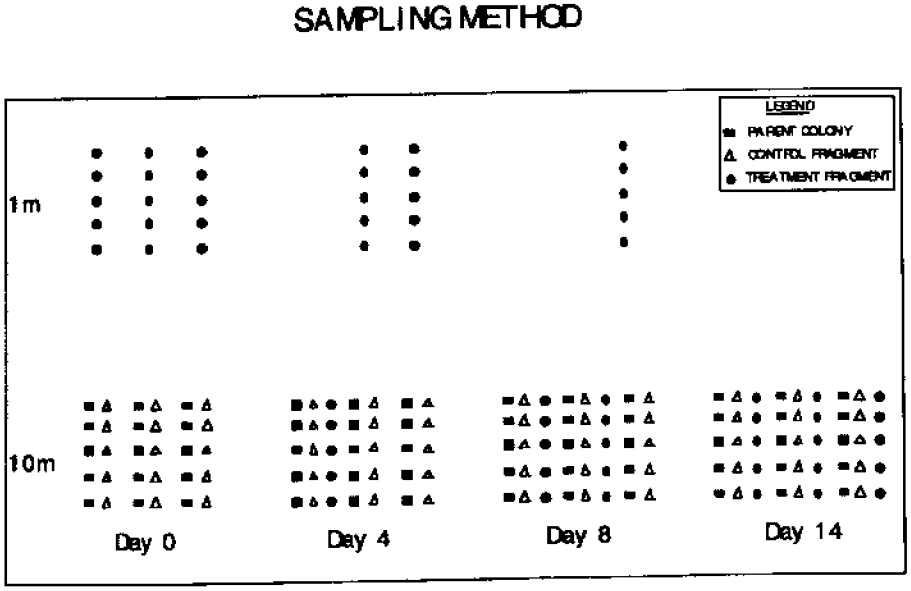

Figure 1. Sampling schedule. Initially (day 0), two fragments each were broken off of each of 15 parent colonies at 10 m (squares = parent colonies). One fragment was transplanted to 1 m (circle = treatment fragment) and the other fragment was placed directly beside the parent colony as a control for transplantation (triangles = control fragments). After 4 days, 5 randomly chosen treatment fragments were returned to 10 m. The same process was repeated on day 8 and day 14. At each time interval, both fragments and the parent colonies were sampled and chlorophyll *a* and total lipid levels were determined.

Two subsamples from each fragment and parent colony were taken by drilling with a 1.25 cm cork borer through the coral plate. One subsample was analyzed for chlorophyll *a* concentrations and the other for total lipid levels in order to determine initial levels of both of these parameters. Since lipid and chlorophyll *a* levels did not vary significantly within a coral plate (determined prior to conducting the experiment), a single sample from each fragment and parent colony adequately

represented the concentrations of these parameters ($F = 2.167$, $n = 6$, $p < 0.08$; $F = 1.596$, $n = 6$, $p < 0.194$ respectively).

After four days (July 16), all of the treatment fragments, control fragments and the parent colonies were again sampled and analyzed for total lipid and chlorophyll *a* levels. Five of the fifteen treatment fragments were randomly selected, returned to their original sites at 10 m and subsequently monitored for signs of recovery. This procedure was repeated again on days 8 and 14 (July 20 and July 26) of the experiment thus returning all treatment fragments to their original depth (Fig. 1).

Chlorophyll *a* and lipid analyses were performed as follows: Chlorophyll *a* was extracted from fresh, finely-ground samples according to the method described by Jeffrey and Humphrey (1975) and reported in ug/cm^2. Lipids were extracted from finely-ground samples (samples had been frozen at - 50°C for 1- 2 weeks prior to extraction) in a chloroform:methanol (2:1,v:v) solution. Extracts were then washed once with 0.88% potassium chloride solution, three times with a methanol:water solution (1:1,v:v) and dried at 50°C for 24 hours before weighing. Animal tissue biomass was determined following lipid extraction by burning the skeleton and remaining tissue residue in a muffle furnace at 450°C for 6 hours. Lipid content in corals was reported as % lipid per gram dry tissue weight. This method differs slightly from Harland *et al.* (1992) where samples were decalcified prior to lipid extraction which can result in lipid loss during the decalcification process (triglycerides can hydrolyze in acid solutions and the glycerol component of the molecule is then soluble).

The data were analyzed by pairwise comparisons between parent colonies and control fragments as well as between control fragments and treatment fragments to determine if lipid and/or chlorophyll *a* levels had changed in treated fragments. The null hypothesis was that the difference between the above mentioned pairs was less than or equal to zero and was rejected at an alpha level of 0.05 by means of a paired ANOVA on each sampled date. Comparisons of the lipid and chlorophyll *a* levels in the treatment fragments that had recovered for 10, 6 and 0 days were examined by means of an ANOVA. The relationship between lipid and chlorophyll *a* levels was assessed by means of a correlation analysis.

RESULTS

Both the parent colonies and the control fragments were observed to have normal coloration throughout the experiment. Their chlorophyll *a* levels were not significantly different from each other at any time (Table I)(Fig. 2). Treatment fragments were initially observed to have similar pigmentation as the controls but began to pale on the third day of exposure to high light (Table I). They became progressively paler with increasing exposure time. Chlorophyll *a* levels were significantly lower in treatment fragments than in control fragments on days 4, 8 and 14 (Table I)(Fig. 2).

During this interval, lipid levels in the parent colony, control fragment and treatment fragment samples did not differ (Table 1)(Fig. 2). Further indication that bleaching, as indicated by decreased chlorophyll *a*, was not accompanied by a decrease in lipid levels was revealed by a lack of a significant correlation between chlorophyll *a* and the percent lipid per gram dry weight ($F=1.734$, $n=171$, $p<0.190$) (Fig. 3).

Recovery in bleached fragments was assessed by directly comparing lipid and chlorophyll *a* levels in fragments that had bleached and recovered for 4 and 10 days, 8 and 6 days and 14 and 0 days, respectively. Lipid levels and chlorophyll *a* levels were not significantly different between treatment fragments that had recovered for 10, 6 and 0 days ($F=0.124$, $p<0.884$, $n=15$; $F=0.243$, $p<0.788$, $n=15$ respectively).

Table I: Results of pairwise comparisons. CHL *a* = chlorophyll *a*, P-CT = pairwise comparison between parent colonies and control fragments, CT-T = pairwise comparison between control fragments and treatment fragments, n = number of samples.

	DAY 0		DAY 4		DAY 8		DAY 14	
	P<	n	P<	n	P<	n	P<	n
CHL *a*								
P-CT	0.78	14	0.28	15	0.91	10	0.44	4
CT-T	0.38	14	0.00	15	0.00	10	0.04	4
%LIPID								
P-CT	0.98	14	0.63	15	0.26	10	0.55	4
CT-T	0.53	14	0.33	15	0.30	10	0.19	4

DISCUSSION

The upper surface of all treatment fragments appeared paler in color after 3 days of bleaching and remained pale throughout the experiment. This overall paler appearance was reflected in lower chlorophyll *a* levels. The decrease in pigment levels did not recover to pre-bleach levels during the course of the experiment irrespective of the length of exposure to high light. Lipids did not decrease in bleached fragments of *M. verrucosa* over the course of two weeks. Rather, a general trend towards increased lipid levels in the parent colony, control fragment and the treatment fragment was observed. *M. verrucosa* has a natural lunar cycle to its lipid levels that corresponds to spawning (Stimson, 1987). Since the experiment was initiated immediately following the July spawning, the observed trend in increased lipids seems to be a reflection of this natural cycle of reproduction. The lack of a significant correlation between chlorophyll *a* and the percent lipid per gram dry weight is consistent with the observation that decreases in chlorophyll *a* were not accompanied by decreases in lipid levels (Fig. 3). Studies by Fitt *et al.* (1993) showed that lipids in 3 bleached *Montastrea annularis* colonies were lower than in 3 unbleached colonies 6 months following bleaching. There are several possible reasons for the discrepancy between this study and that of Fitt *et al.* (1993). Lipids may be metabolized very slowly in bleached corals making decreases in lipid levels apparent only long after initial bleaching. However, shading experiments by Harriott (1993) indicated that lipid levels in the Hawaiian coral *Pocillopora damicornis* decreased in just one week. When corals were shaded, zooxanthellae were initially unable to maintain photosynthesis at the same level as when in full sunlight. Under these conditions, lipid stores were metabolized in order to fulfill the corals' daily energetic demands. This situation is analogous to bleaching in that bleached corals suffer from decreased photosynthetically-derived carbon as well. Based on this evidence, one might expect to observe a change in lipid levels in the first week of bleaching. But *M. verrucosa* is a much slower growing species than *P. damicornis* and predictions of a rapid response in *Montipora* based on the latter coral may be unrealistic. *Montipora verrucosa* in Hawai'i lives in a more extreme habitat than Caribbean corals. Therefore it may be naturally more capable of coping with stresses such as bleaching making it difficult to detect any lipid responses after a short bleaching period. *M. verrucosa* spawns every month during the summer. Eggs released during spawning can be up to 70% lipid by dry weight (Arai *et al.*, 1993). If stressed corals can resorb lipids from unreleased eggs, or can delay ripening eggs for spawning, then decreases in total lipid levels could possibly only be detectable over a longer period of time.

Corals may increase heterotrophy in order to supplement their nutritional demands. However, it is suspected that heterotrophy may only account for 10% of the corals diet in some species (Wellington, 1982). Lipid levels simply do not change when corals are bleached. In this study treated fragments were compared with genetically identical control fragments in a pairwise fashion. This is a very robust experimental method because it controls for genetic variation between corals. Paired comparisons such as these have not been used in any previous experiments which examined lipids in corals. I believe that my results are convincing evidence that lipids do not change within the first two weeks of bleaching. The hypothesis that lipid levels in

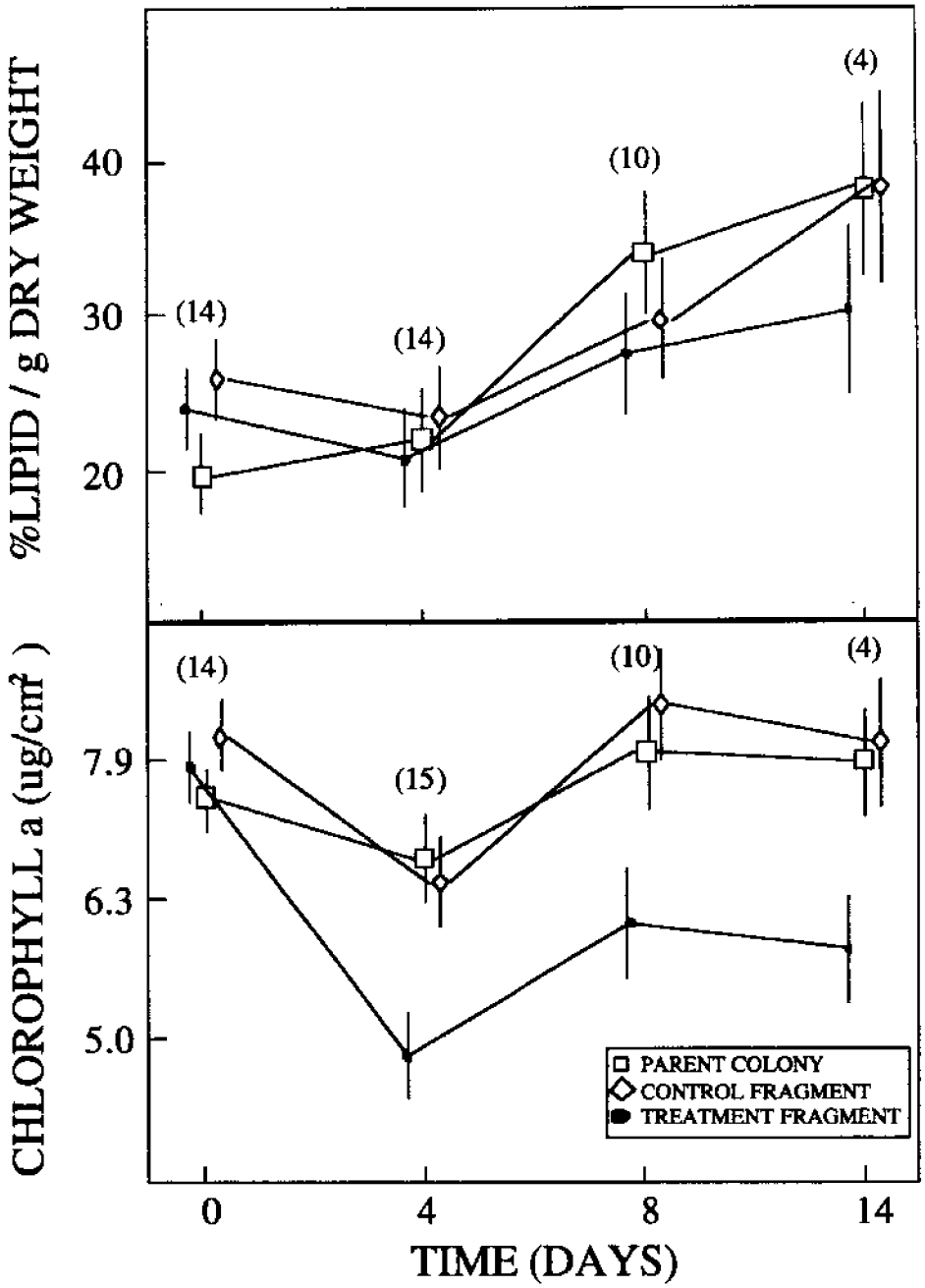

Figure 2. Average %lipid per gram dry weight (± one standard error) and average chlorophyll *a* (ug/cm^2) (± one standard error) on day 0, 4, 8 and 14. Open squares, open diamonds and solid circles represent parent colonies, control fragments and treatment fragments, respectively, and are offset slightly from one another so that error bars do not overlap. The number of parent colonies, control fragments and treatment fragments are indicated in parentheses.

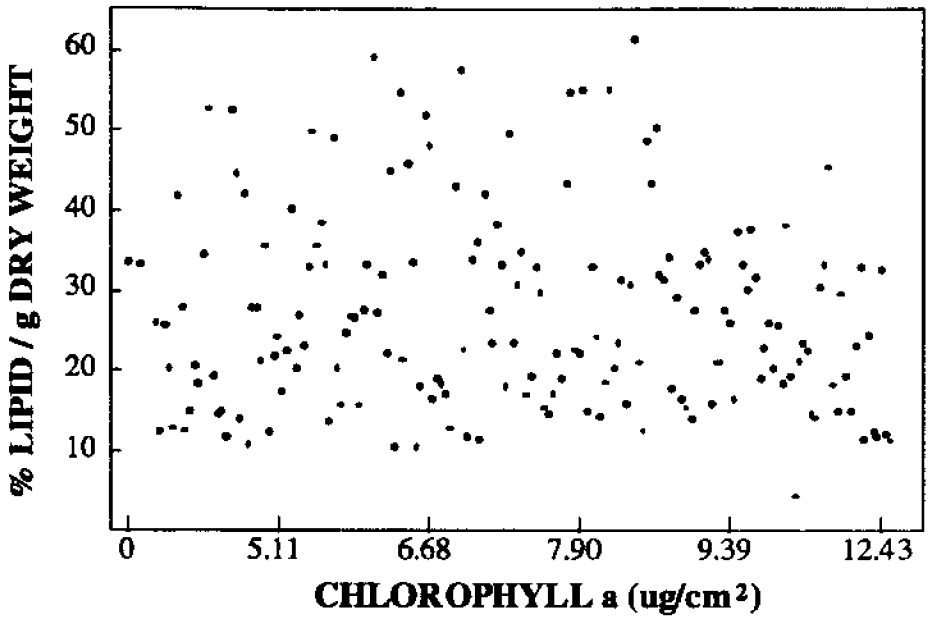

Fig. 3. Correlation between % lipid per gram dry weight and log chlorophyll *a* (ug/cm^2).

recently bleached corals would be lower, and that lipid content in corals would decrease as the length of the bleaching period increased, was rejected. No lipid response was detectable in bleached fragments over the course of the initial 2 weeks. Running the experiment for 1, 2 and 3 months would determine whether or not lipid levels change in bleached corals over a longer period of time. As well, monitoring gamete production and release over the same period in bleached and unbleached corals would reveal whether or not gametes are being resorbed or being prevented from developing during bleaching.

After natural bleaching events such as those observed in the Caribbean in 1983 and 1987, some coral species recovered more rapidly and more frequently than others. The ability to withstand and recover from prolonged bleaching events (i.e., several months) may yet be related to the amount of lipid stores, and may yield some insight into why some coral species recover more quickly from bleaching events than others. However, lipids do not appear to play a role in short-term coral bleaching and recovery.

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Uneven bleaching within colonies of the Hawaiian coral *Montipora verrucosa*

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ABSTRACT: When a coral colony undergoes bleaching, the response may not be uniform across the entire colony. In this study, the bleaching response of *Montipora verrucosa* colonies varied significantly depending on the spectral quantity and quality of solar irradiance that each area of the colony was exposed to. In order to induce bleaching, five *M. verrucosa* colonies were transplanted from a low-light environment at 10 m to a high light environment at 1 m. Four different light treatments were concurrently imposed on four distinct regions of each coral fragment. The four treatments included 1) elevated levels of PAR (photosynthetically active radiation: 400 - 700 nm), 2) elevated levels of PAR and UV-A (320 - 400 nm), 3) elevated levels of PAR, UV-A and UV-B (300 - 320 nm), and 4) a control treatment with PAR, UVA and UVB screened to levels that mimicked radiation levels found at 10 m. At the completion of the nine day treatment period, bleaching intensity was quantified by measuring the concentration of chlorophyll *a*, zooxanthellae density, and percent lipid per gram dry weight in samples from each treatment region of each coral fragment. Chlorophyll *a* per zooxanthella was also calculated. Chlorophyll *a* concentrations decreased significantly in the two treatments that included elevated levels of PAR and UV ($p < 0.013$). Neither zooxanthellae densities nor lipid levels decreased significantly in any of the treatments. These results support the hypothesis that corals do not bleach uniformly when treated with different levels of solar irradiance. It also appears that *M. verrucosa*, when induced to bleach in this manner, responds by decreasing chlorophyll *a* concentrations, and not by expelling zooxanthellae.

INTRODUCTION

Pigment loss in scleractinian corals due to reduction in zooxanthellae density and/or the loss of photosynthetic pigment per zooxanthella cell is a phenomenon known as coral bleaching. Over the past 15 years, the incidence of widespread bleaching events on coral reefs has increased throughout the world. Elevated temperature, ultraviolet radiation, total solar irradiance and sedimentation are among the environmental factors which have been found to cause bleaching in corals (Gleason & Wellington,1993; Jokiel & Coles, 1990; Hoegh-Guldberg & Smith, 1989). Bleaching can result in the interruption of coral growth, reduction in reproductive output and, eventually, death (Jokiel & Coles, 1977; Glynn & D'Croz, 1990; Szmant & Gassman, 1990). The gravity of this phenomenon has lead to increased research examining the effects of bleaching on coral biology and ecology.

Uneven bleaching within a coral colony has been observed by several researchers (Fitt et. al., 1993; Jokiel & Coles, 1990). Jokiel and Coles (1990) stated that "portions of coral colonies receiving the highest incident radiation bleach more readily than portions that are shaded." In a paper by Fitt *et al.* (1993), a large color photograph of a bleached caribbean coral, *Montastrea annularis* , clearly illustrates a mottled bleaching pattern. However, these studies did not quantitatively address the issue. We also observed uneven bleaching in experimentally manipulated *Montipora verrucosa* coral fragments. Each fragment had a wire identification tag wrapped around it and was induced to bleach via transplantation from a low-light site at 10 m to the high-light site at 1 m for one week. Initially, the coral fragments appeared to have bleached uniformly. Closer examination revealed that the area shaded by the wire tag was much darker than the adjacent, unshaded area. Following this observation, we designed a study to empirically measure differential bleaching response within a coral colony by simultaneously exposing different areas of the plating coral, *M. verrucosa*, to varying levels of solar irradiance. We tested the hypothesis that uneven bleaching within a coral colony occurs as a result of different levels of incident solar radiation. Manipulations were also performed in order to determine which portion of the irradiance spectrum was inducing the response: PAR, UV-A, UV-B, or some combination of the three.

MATERIALS AND METHODS

The experiment was conducted between July 23 and August 1, 1994, at the Lighthouse Point (LP) and the Bridge to Nowhere (BTN) sites on Coconut Island, Hawai'i. A coral

fragment with minimum dimensions of 30 cm x 10 cm was broken off from each of five separate colonies of the plating morphology of *Montipora verrucosa*, at a depth of 10 m at the LP site. A smaller subfragment with dimensions approximating 100 cm^2 was then broken off each larger fragment, tagged and placed back at the site of origin as a control for transplantation. The five larger fragments were exposed to increased light levels via transplantion to a depth of 1 m at the BTN site, and each centrally placed under a separate treatment frame for nine days (Fig. 1). Each frame was 50 cm x 50 cm and consisted of four adjacent treatment bands (Fig. 1):

1) A control treatment with integrated irradiance levels between 300 and 700 nm, similar to those found at 10 m (50 cm x 20 cm band of ultraviolet radiation-transparent (UVT) Plexiglas overlain with two layers of neutral density filter).
2) A high PAR + UV-A + UV-B treatment (300 - 700 nm) (a 50 cm x 5 cm band of UVT Plexiglas).
3) A high PAR + UV-A treatment (320 - 700 nm) (a 50 cm x 5 cm band of UVT Plexiglas overlain with UV-B opaque mylar).
4) A high PAR treatment (400 - 700 nm) (50 cm x 20 cm band of UV-opaque Plexiglas)[1]..

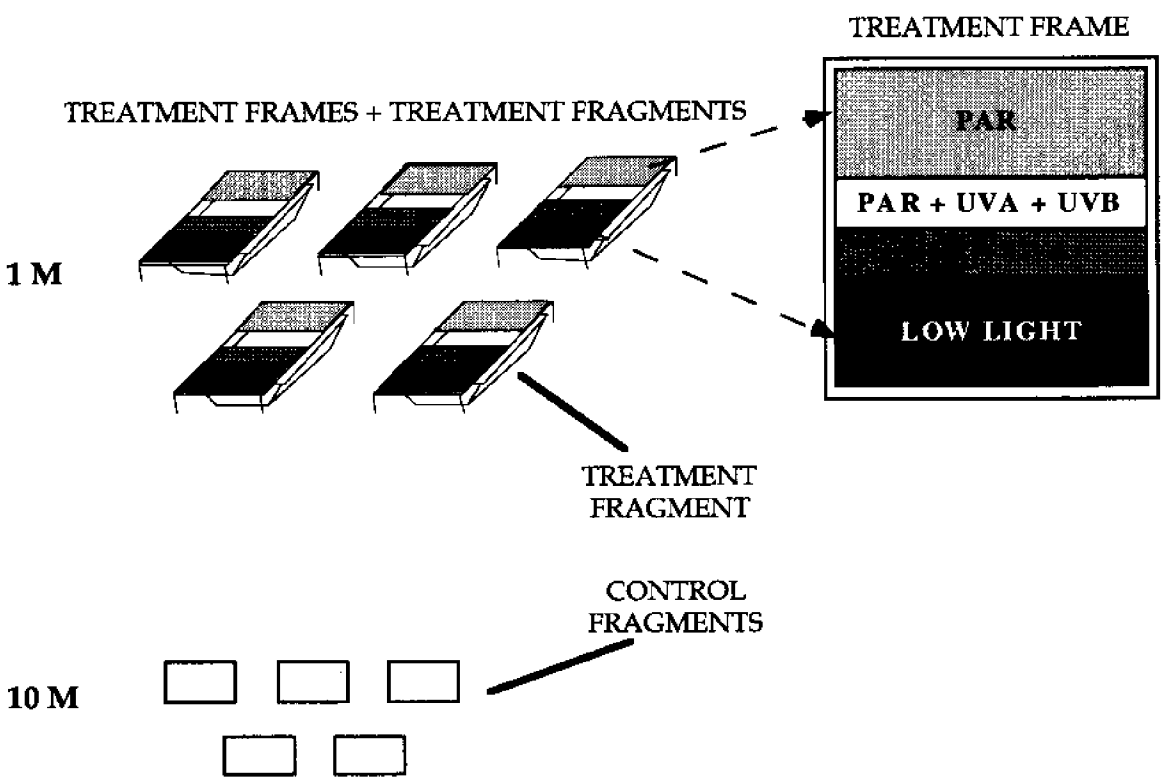

Figure 1: Summary of methodology. Uneven bleaching was induced in Hawaiian *Montipora verrucosa* coral fragments by transplanting them from 10 m (low-light) to 1 m (high-light) and centrally placing them under a treatment frame for 9 days. Each frame consisted of a PAR, PAR + UV-A + UV-B, PAR + UV-A transparent strip as well as a low-light control which reduced total irradiance (PAR + UV-A + UV-B) by 70% in order to simulate the lower light levels at 10 m.

By placing each coral fragment under a separate treatment frame, different areas of each coral's surface were simultaneously exposed to the four different treatments. Transplanted fragments and treatment frames were affixed to a uniform substrate plastic mesh platform.

[1] See Gulko *et al.* (this volume) for scans and more information on the use of these filters.

Average total irradiance at the high-light site (BTM at 1 m) was 70% greater than that observed at the 10 m LP site (based on integrated light measurements made every 2 nm using a Li-Cor Li-1800UW underwater spectroradiometer between 300-700 nm). Integrated UV-B (300-320 nm), UV-A (320-400 nm) and photosynthetically active radiation (PAR 400-700 nm) levels were approximately 99%, 93% and 66% greater at 1 m than at 10 m respectively. Treatment fragments were transplanted to the BTN site to replicate the low wave action conditions present at 10 m at the LP site (LP site is near a dock with boat traffic). These sites are separated by approximately 100 m, occur in the same small lagoon, and have similar sedimentation and temperature regimes. Sediment was brushed off of the treatment frames daily for the duration of the experiment. Hourly temperature values were recorded both at the 1 m BTN and at the 10 m LP site using two $HOBO_{TM}$ miniature data loggers. The two sites differed by less than 1°C, on average, and neither temperature regime was high enough to induce bleaching (LP average temp. = 26.47 ± 1.98°C and BTN average temp. = 26.95 ± 1.01°C)(Jokiel & Coles, 1977).

After nine days, eight samples were taken from each of the four treatment bands within each coral fragment (for a total of 32 subsamples per fragment) and from the control fragment by drilling with a 1.25 cm^2 cork borer through the coral plate. Three samples were analyzed for chlorophyll *a* concentrations, three for total lipid levels and two for zooxanthellae concentrations per treatment per coral.

Chlorophyll *a* was extracted twice from fresh, finely ground samples in 10 ml of 100% acetone at 4°C for 24 hours. Samples were then centrifuged for 10 minutes. The absorbance of the supernatant was measured using a spectroradiometer and the chlorophyll *a* concentrations were calculated according to the method described by Jeffrey and Humphrey (1975) and reported in ug/cm^2.

Lipids were extracted from finely ground samples (samples had been frozen at -50°C for 1-2 weeks prior to extraction) in a chloroform:methanol (2:1,v:v) solution. Extracts were then washed once with 0.88% potassium chloride solution, three times with a methanol:water solution (1:1,v:v) and dried at 50°C for 24 hours before weighing. Animal tissue biomass was determined following lipid extraction by burning the skeleton and remaining tissue residue in a muffle furnace at 450°C for 6 hours. Lipid content in corals was reported as % lipid per gram dry tissue weight. This method differs slightly from Harland *et al.* (1992) where samples were decalcified prior to lipid extraction which can result in lipid loss during the decalcification process (triglycerides can hydrolyze in acid solutions and the glycerol component of the molecule is then soluble).

In order to determine zooxanthellae concentrations, fresh samples were simultaneously decalcified in 10 ml of 10% acetic acid, preserved with a few drops of 4% formalin and stained with a few drops of Lugol's solution. Once decalcification was complete, samples were centrifuged on "high" setting for 10 minutes, the excess liquid was decanted off and the remainder was homogenized for 30 seconds before being resuspended into 10 ml of 4% formalin for long-term preservation. Four subsamples from each sample were counted using a 0.1 mm^3 hemocytometer and reported as the average number of zooxanthellae per cm^2. The amount of chlorophyll *a* per zooxanthellae was determined by dividing the amount of chlorophyll *a* per cm^2 by the total number of zooxanthellae per cm^2 and was reported in ng of chlorophyll *a* per zooxanthellae.

The mean lipid, chlorophyll *a*, zooxanthellae or chlorophyll *a* per zooxanthella levels for the transplanted control fragment and the low light control fragment were analyzed using a student's t-test. All of the data were then analyzed by pairwise model I ANOVA's between the control and the four treatments to determine if either lipid, chlorophyll *a*, zooxanthellae or chlorophyll *a* per zooxanthella levels had changed in any of the treatments. In all cases the null hypothesis was rejected at an alpha level of 0.05.

RESULTS

On the first day of the experiment, all of the coral treatment and control fragments were uniformly dark brown in color. The portion of the coral treatment fragments positioned under the PAR + UV-A + UV-B, PAR + UV-A and PAR treatment bands began to visibly bleach after the fourth, fifth and seventh day respectively. At the end of the nine day experiment, the coral area under the low-light, PAR + UV-A + UV-B, PAR + UV-A and PAR treatment bands, were dark brown (like the control fragment), almost white, extremely light brown and medium brown in color respectively.

The control and low-light treatment were not significantly different from each other with respect to any of the variables measured. This indicated that breaking off a coral fragment had no significant effect and that changes in the proportionate amounts of PAR, UVA and UVB due to transplantation were negligible. Only changes in spectral quantity and quality had an effect on the manipulated coral fragments.

The degree of bleaching was determined by measuring the chlorophyll *a* and zooxanthellae concentrations. Relative to the control and the low-light treatment, a significant decrease in chlorophyll *a* was observed in the PAR + UVA and PAR + UVA + UVB treatments ($F = 4.894$, $p < 0.013$)(Fig. 2a).

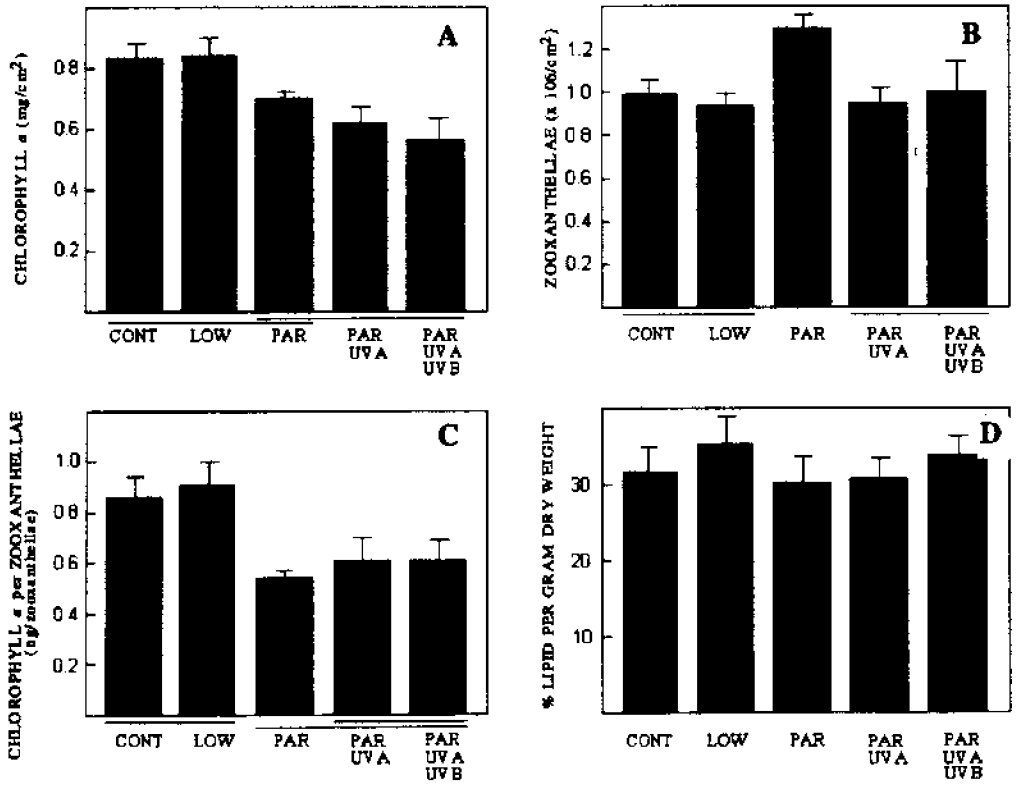

Figure 2: Mean (±SE) A. chlorophyll *a* (mg/cm^2), B. zooxanthellae (x10^6 /cm^2), C. chlorophyll *a* per zooxanthella (ng/zooxanthellae) and D. percent lipid per gram dry weight levels in each of the four treatment regions and control fragments of the Hawaiian coral, *Montipora verrucosa*, after 9 days. Treatments underlined with lines at the same level were not significantly different (alpha = 0.05). CONT = control fragment, LOW=low light treatment, PAR Treatment = PAR (400 - 700 nm), PAR + UV-A Treatment = PAR + UV-A (320 - 700 nm), PAR + UV-A + UV-B Treatment = PAR + UV-A + UV-B (300-700 nm).

These decreases in pigment levels in the PAR + UV-A and PAR + UV-A + UV-B treatments were not accompanied by any changes in zooxanthellae concentrations. Zooxanthellae levels were not significantly different in the PAR + UVA and PAR + UVA + UVB treatments relative to the control and low-light treatments. However, zooxanthellae concentrations were significantly higher in the PAR treatment ($F = 3.324$, $p < 0.047$)(Fig. 2b). Consequently, the chlorophyll *a* per zooxanthella levels were significantly lower in the PAR treatment ($F = 4.088$, $p < 0.025$)(Fig. 2c).

Energy reserve levels were determined by measuring lipid levels. Lipid levels did not differ significantly between any of the treatments or control ($F = 0.351$, $p < 0.789$)(Fig. 2d).

DISCUSSION

Chlorophyll *a*, zooxanthellae and chlorophyll *a* per zooxanthella levels varied significantly in response to various light conditions within fragments of the coral, *Montipora verrucosa*. The portion of the coral fragments exposed to elevated levels of PAR + UVA + UVB, PAR + UVA, and PAR exhibited high- to low-levels of bleaching respectively. There was no bleaching in either the low-light control or the transplantation control.

The portions of the solar spectrum which induced the bleaching response were elucidated. While pigments levels did decrease in all elevated irradiance treatments (PAR, PAR + UVA, PAR + UVA + UVB), significant decreases were only detected in the two treatments that included ultraviolet radiation (Fig. 2a). Under elevated PAR conditions (UV excluded), *M. verrucosa* did not significantly lose chlorophyll *a* relative to controls, but the density of zooxanthellae increased, resulting in an overall decrease in the calculated value of chlorophyll *a* per zooxanthella (Fig. 2a, b, c). This evidence indicates that in Hawaiian *M. verrucosa*, PAR, as well as UV, causes chlorophyll *a* to decrease but that under elevated PAR conditions alone, the coral may be able to compensate for this by increasing the number of zooxanthellae. Perhaps the increase in zooxanthellae density in the absence of UV is a response to an increase in potential light harvest without an increase in the biologically damaging ultraviolet radiation.

Energy reserve levels were determined by measuring lipid levels. The percent lipid content per gram dry weight did not significantly differ between any of the treatments or control conditions. Under standard physiological conditions, fatty acids and glycerol are synthesized by zooxanthellae from photosynthetically-fixed carbon and translocated to the host where they are either metabolized or transformed and stored primarily in the form of wax esters and triglycerides (Batey & Patton, 1984). When corals bleach, chlorophyll *a* levels decrease; hence the amount of carbon fixed also potentially decreases and other sources of carbon have to be relied upon. Despite a dramatic decrease in photosynthetic pigment (chlorophyll *a*) in the bleached portions of the coral colonies, lipid reserves did not decrease in *M. verrucosa*. Other work by Grottoli-Everett (1995) shows that lipid levels in *M. verrucosa* do not change even after two weeks of bleaching. Perhaps the zooxanthellae are able to maintain a high level of fixed carbon production because at high-light levels, the chlorophyll *a* pigments are being fully saturated. Alternatively, decreased metabolism, increased heterotrophy, gametes resorption or some combination of these factors during the early stages of bleaching are mechanisms by which bleached corals may be compensating for decreased photosynthetically-derived, fixed carbon.

Given that varying degrees of bleaching occurred in the three elevated irradiance treatments, and not in the low-light control treatment within fragments of *M. verrucosa*, we accept our hypothesis that uneven bleaching within a coral colony occurs as a result of different levels of solar irradiance. The results of this experiment suggest that PAR, UV-A and UV-B have a synergistic effect on bleaching in *M. verrucosa*, as the decrease in chlorophyll *a* concentration was greatest when all three sections of the spectrum were allowed through the filters, and respectively less in the treatments where UVB was screened out and the treatments where no UV was allowed through the filters. Furthermore, this study indicates that bleaching due to increased solar irradiance in the Hawaiian coral, *M. verrucosa*, results from a decrease in chlorophyll *a* per zooxanthella and not from a decrease in the number of zooxanthellae.

Differential bleaching responses within a coral colony are quantifiable. While uneven bleaching has been mentioned previously in the literature, this study is the first documented empirical evidence of this observation. When conducting experiments on bleached coral, researchers must be careful to take into account the heterogeneity involved in bleaching in order to avoid biased sampling.

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The metabolic response of *Fungia scutaria* to elevated temperatures under various UV light regimes

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ABSTRACT: Photosynthesis and respiration of *Fungia scutaria*, acclimatized to UVO and UVT treatments, were measured at 27°C and 29°C. In the summer, average seawater temperatures in Kane'ohe Bay, O'ahu, Hawai'i range between 26 - 27°C. At 29°C, the photosynthetic rates for coral acclimatized to UVO, UVT, and UVT/UVO conditions decreased by 71%, 10% and 24%, respectively, from measurements at 27°C. Chlorophyll *a* and c_2, on the other hand, remained constant at both temperatures and between all three treatments.

INTRODUCTION

There has been an increased concern over the amounts of solar ultraviolet radiation that reaches the earth's surface, in particular at the equator where ozone is naturally the thinnest. Some evidence implies that UV radiation levels have increased due to a decrease in the ozone layer. In clear tropical waters UV radiation, especially UV-B, penetrates to deeper depths due to the low levels of dissolved organic matter and chlorophyll found in the water (Smith & Baker, 1979). Due to a reported increase in the occurrence of coral bleaching, which results in a decrease in either chlorophyll or algal density, researchers have tested the effects of UV on coral. Although temperature, salinity, and sedimentation are other possible factors contributing to bleaching, UV radiation has been found to affect coral growth, calcification, reproduction and viability (Jokiel & York, 1982; Jokiel & Coles, 1990; Szmant & Gassman, 1990; Gleason & Wellington, 1993).

There are many possible mechanisms that animals can protect themselves from UV damage. They range from behavioral adaptations, such as moving into low UV environments, to the enhanced enzyme activity of catalase and ascorbate peroxidase in symbiotic algae and superoxide dismutase in both the host and algal symbiont (Shick *et al.*, 1991). In addition, coral and/or their algae may also show an increase in the production and/or accumulation of UV absorbing compounds like mycosporine-like amino acids (Dunlap & Chalker, 1986). For example, Dunlap *et al.* (1986) discovered that various *Acropora* species contained more UV-absorbing compounds at shallower depths than their deeper counterparts. Furthermore, Drollet *et al.* (1993) found that *Fungia fungites* secreted mucus containing MAAs. The concentration of these MAAs decreased with increasing depth as the levels of UV radiation decreased. Thus, these coral appear to be adapting to their shallow environment.

Past research has also shown a synergistic effect between many factors including light, temperature, salinity, and ultraviolet radiation (Coles and Jokiel, 1978; Hoegh-Guldberg and Smith, 1989; Lesser *et al.*, 1990). It appears that a combination of any of these factors generally results in a stress response that would not be observed if there were only one factor involved. The purpose of this study was to determine whether *Fungia scutaria* exposed to UV radiation would be more susceptible to increasing temperatures, and thus cause coral bleaching at a lower temperature.

MATERIALS AND METHODS

Collection and Experimental Design

Specimens of *Fungia scutaria*, 1-3 inches in diameter, were collected from patch reefs in Kane'ohe Bay, O'ahu at depths ranging between 5-10 feet during the month of June, 1994. All individuals were returned to the Hawai'i Institute of Marine Biology, Coconut Island, O'ahu and placed in a running seawater tank. After adjusting to laboratory conditions for several days, *F. scutaria* were acclimatized for twenty-five days in treatments which either blocked or allowed solar ultraviolet radiation. A sheet of Aclar® (Allied Chemical) was used as a ultraviolet-transparent (UVT) filter whereas a sheet of 100% clear Acrylic was used to block all solar ultraviolet radiation (UVO) (see Gulko *et al.*, this volume). After the twenty-five day incubation period, one-half of the individuals exposed to solar ultraviolet radiation were transferred into UVO conditions. These

coral, maintained in UVT conditions for twenty-five days and UVO conditions for seven days, will be categorized as being exposed to a UVT/UVO treatment.

The temperature inside the experimental tank was regulated; however, there existed a regular daily fluctuation. Thus, the temperature was recorded using a Hobo-Temp-XT Data Logger (Fig. 1). Initial temperature during the first twenty-five days was aproximately 27.9°C during the day and 26.9°C at night. At the end of Day 25, the temperature was increased and maintained for seven days at 29.9°C during the day and 27.9°C at night.

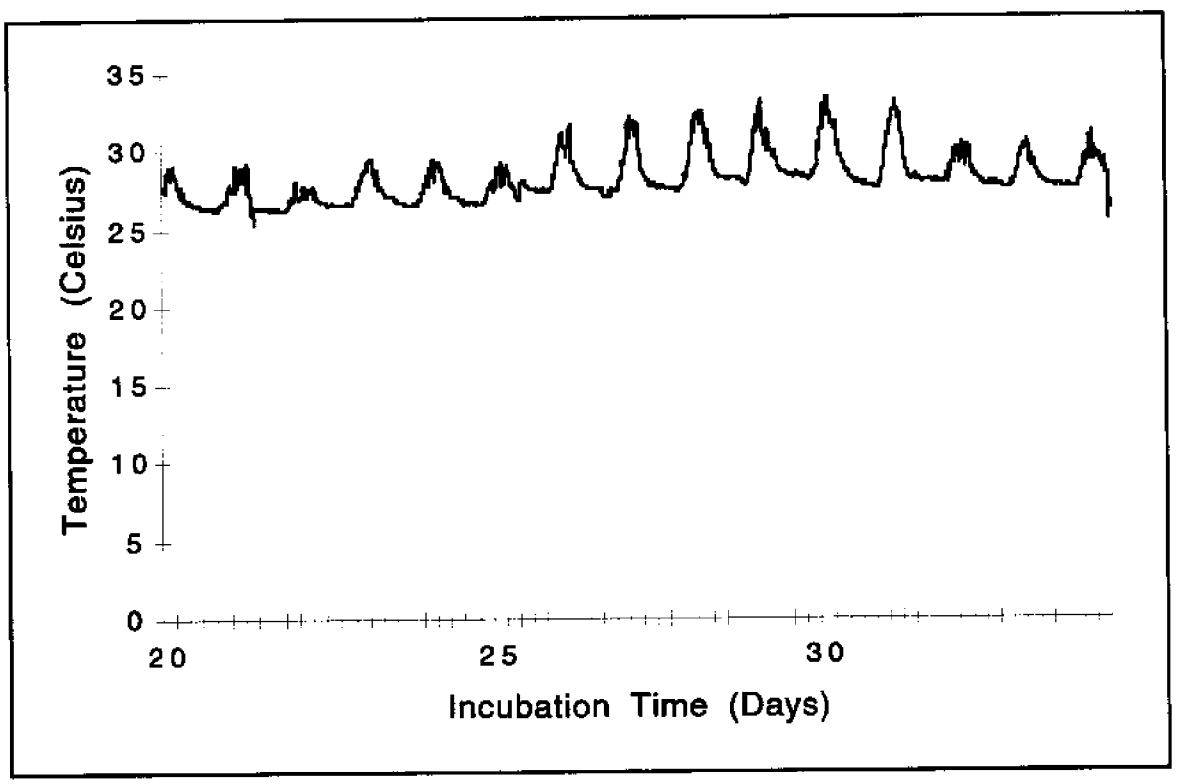

Figure 1. Seawater temperature measured at twelve minute intervals inside the experimental tank at the Hawai'i Institute of Marine Biology, Coconut Island, O'ahu from July 25, 1994 (Day 20) through August 8, 1994 (Day 34). Net photosynthesis and night respirationwere measured at Days 25 and 32.

YSI Electrodes (Model 5739) and Dissolved Oxygen Monitors (Model 58) were used in 880 ml volume chambers that were placed within the experimental tank. Net photosynthetic and night respiratory rates were measured at 27°C for coral exposed to UVO and UVT conditions and at 29°C for coral exposed to UVO, UVT, and UVT/UVO conditions. A total of three corals were used per treatment at each temperature. Both photosynthesis and respiration at each temperature were measured on the same coral. Data acquisition and analysis were obtained using Sable Systems Datacan (Version 4.0). After measuring oxygen evolution and consumption, the displacement volume and wet weight for each coral were determined.

Chlorophyll-*a* Analysis

Two samples taken from each coral were extracted twice in acetone and refrigerated in the dark. The chlorophyll extracts were centrifuged for 10 minutes before their absorbances were read using a Hewlett Packard 8452A Diode Array Spectrophotometer. Chlorophyll *a* and c_2 were calculated using Jeffrey & Humphrey's (1975) equations. The chlorophyll content reproted for each coral is an average of the two samples taken.

RESULTS

Net photosynthesis for coral acclimatized to UVO conditions varied from coral under UVT conditions at both temperatures (Fig. 2). At 27°C the photosynthetic rate was 1.6 times greater for coral maintained under UVO conditions than those under UVT conditions. However, at 29°C the rate of UVT-acclimatized coral was 1.5 times greater than for UVO coral. Unlike both UVO and UVT, UVT/UVT coral showed no difference in rates from the two other treatments.

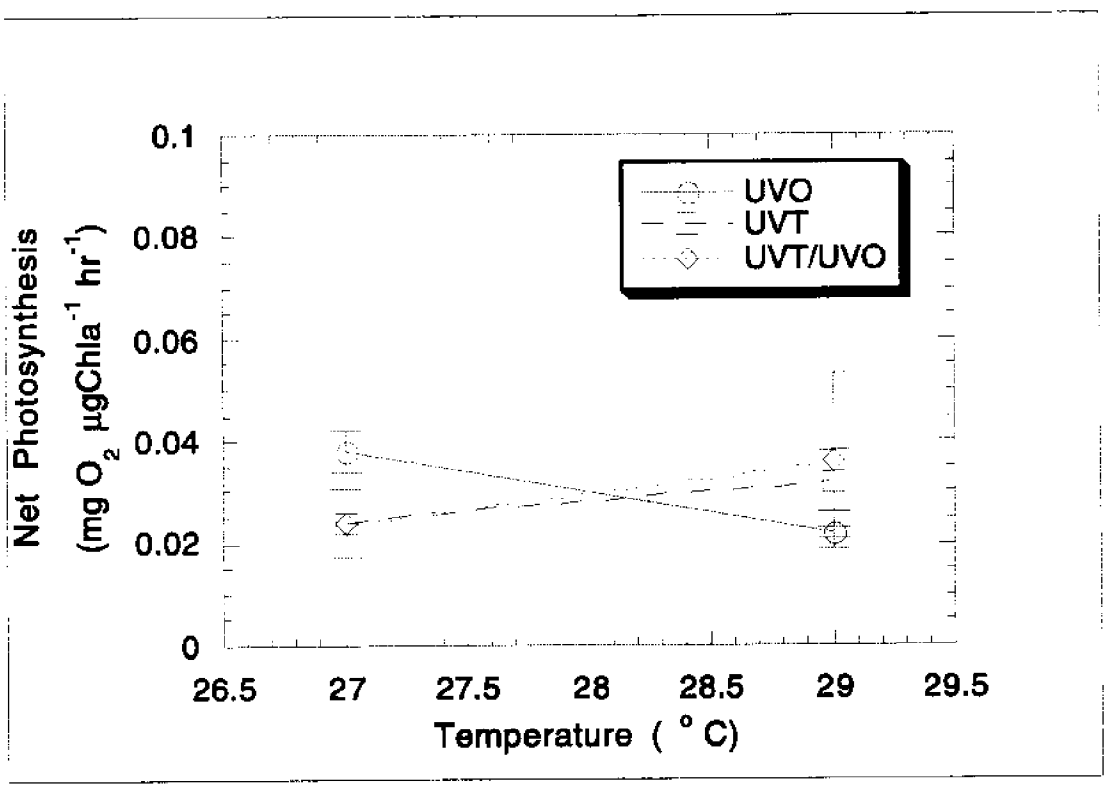

Figure 2. Net photosynthetic rate for *F. scutaria* with increasing temperature for coral either exposed to solar UV, blocked from UV, or exposed to UV initially before being transferred to UV-opaque conditions. Each point represents an average of three coral and bars represent SEM.

Within each experimental treatment, only UVO significantly decreased from 0.05 ± 0.015 mgO_2/g/hr at 27°C to 0.028 = 0.005 mgO_2/g/hr. The two remaining treatments, however, showed a slight increase but the values were not significantly different between the two temperatures.

Dark respiration for UVO acclimatized coral was 1.6 times greater than UVT acclimatized coral (Fig. 3). However at 29°C, there was no evident difference between the three treatments.

P/R ratios for coral acclimatized to UVO, UVT, and UVT/UVO conditions decreased 81%, 15%, and 6%, respectively, from 27 to 29 °C (Fig. 4). Net photosynthesis, rather than dark respiration, appears to account for the lower P/R ratios.

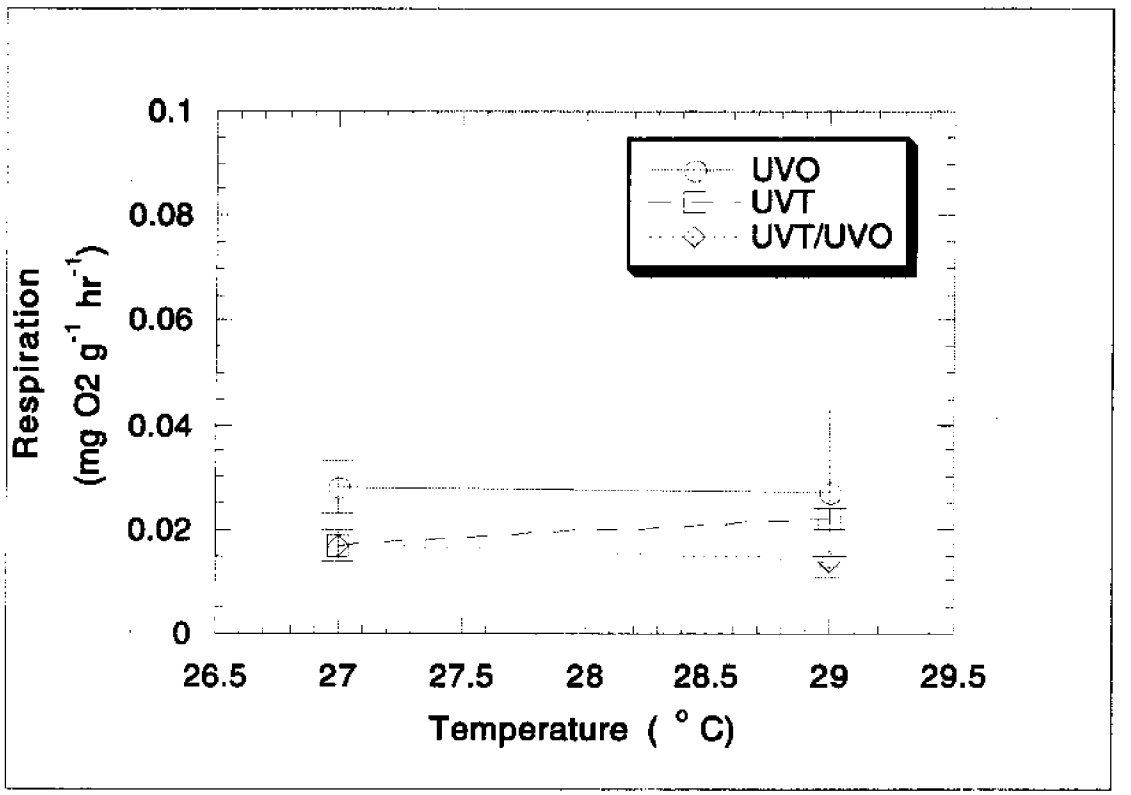

Figure 3. Night respiration for *F. scutaria* with increasing temperature for coral either exposed to solar UV, blocked from UV, or exposed to UV initially before being transferred to UV opaque conditions. Each point represents an average of three coral and bars represent SEM.

Chlorophyll *a* and c_2 concentrations did not vary both between experimental treatments at each temperature and between temperatures within the same treatment. For chl *a*, UVO acclimatized coral contained 53.2 = 9.6 µg and 45.7 = 4.9 µg at 27°C and 29°C, respectively (Fig. 5). Similarly, coral under UVT conditions contained 48.6 = 7.5 µg and 48.4 = 4.0 µg at 27°C and 29°C, respectively, as well as UVT/UVO coral which contained 52.7 = 12.5 µg. Chl c_2 concentrations were lower than chl *a* as is to be expected (Fig. 6). At 27°C, UVO and UVT coral contained 33.4 = 10.1 µg and 29.1 = 7.3 µg, respectively, whereas at 29°C, UVO, UVT, UVT/UVO coral have 21.2 = 2.8 µg, 20.1 = 1.2 µg and 26.7 = 6.3 µg, respectively.

DISCUSSION

The metabolic response of *F. scutaria* seems to indicate that initially, at 27°C, UV has a detrimental effect on UVT-acclimatized coral due to the lower net photosynthetic rate. Since chl *a* and chl c_2 content of these individuals do not vary, UV doesn't affect either the accessory or reaction center pigments; however, it does reduce the photosynthetic efficiency of the coral. Shick *et al.* (1991) observed a similar trend in the octocoral, *Clavularia*, in which UVT acclimatized individuals had a 50% decrease in net photosynthesis compared to individuals acclimatized to UVO conditions even though chlorophyll content remained the same.

At 29°C, the effect of UV on the coral seems to be shadowed by the effect of a 2°C increase in temperature. UVO acclimatized coral photosynthesized the least even though the

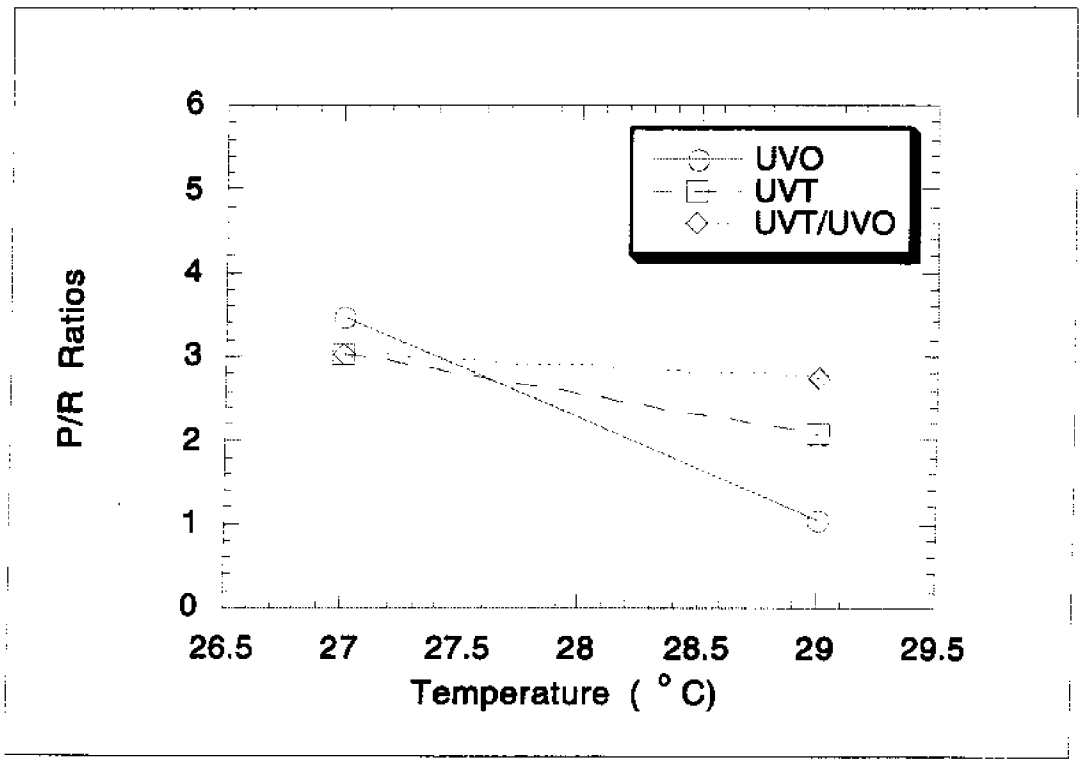

Figure 4. P/R ratios for the three UV treatments at 27°C and 29°C.

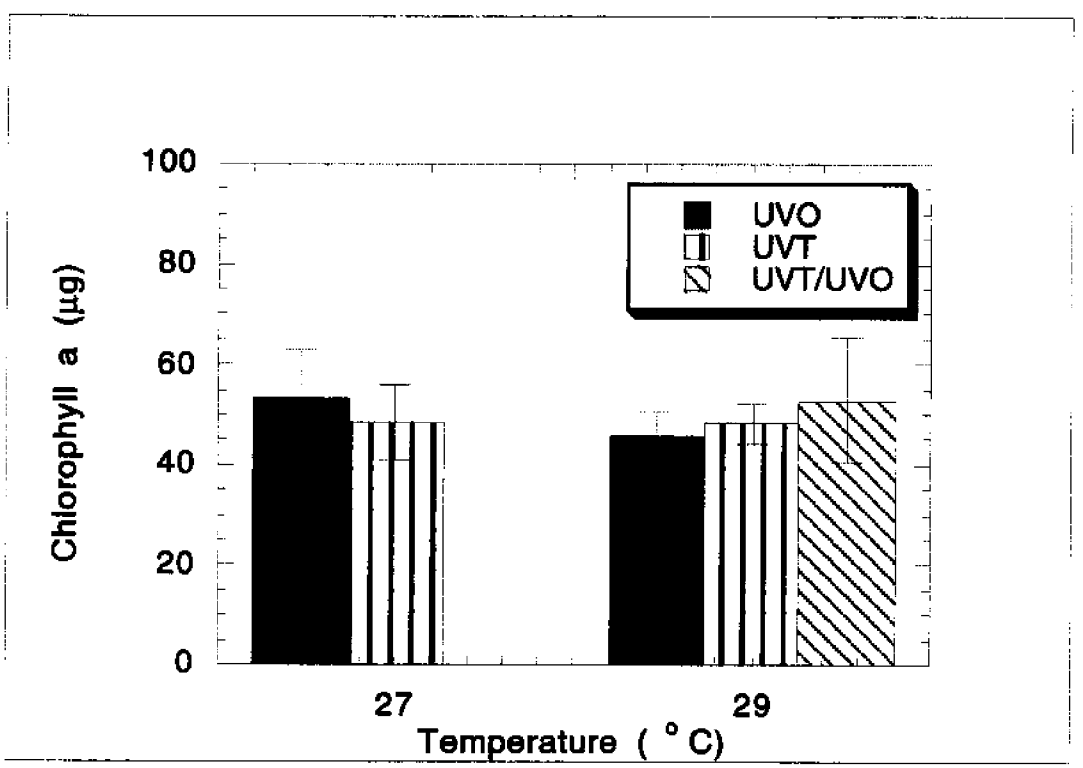

Figure 5. Chlorophyll *a* concentrations for coral acclimatized to the three experimental treatments. Each value represents and average of two samples taken from each coral (n=3). Bars represent SEM.

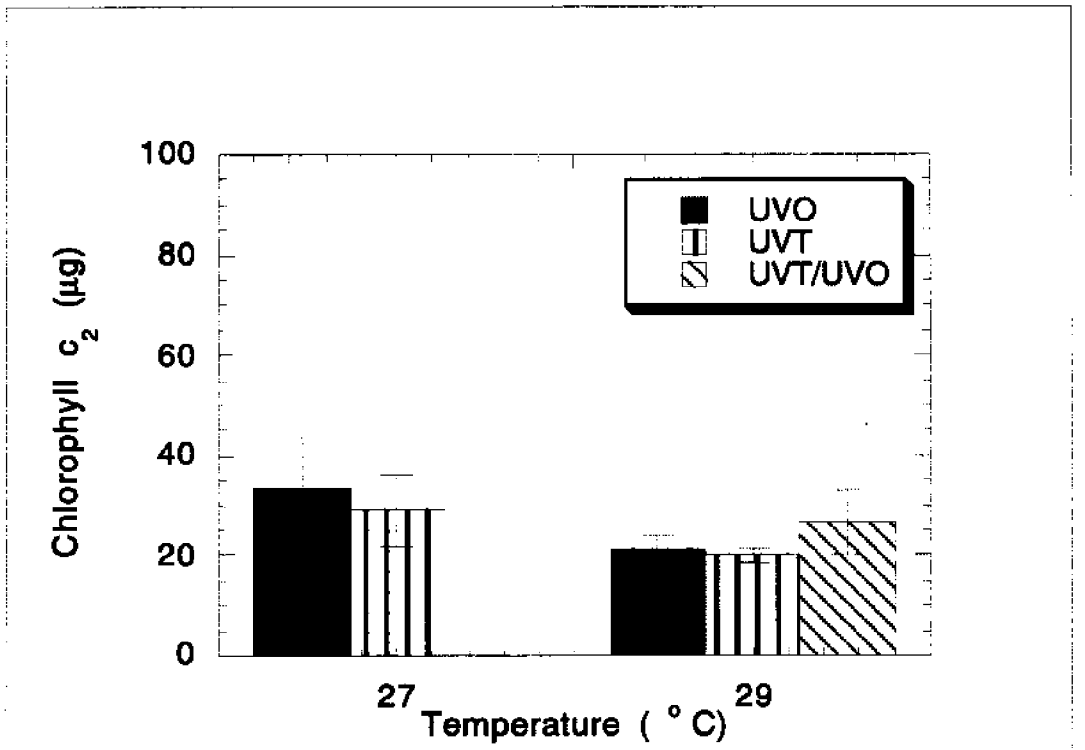

Figure 6. Chlorophyll c_2 concentrations for coral acclimatized to the three experimental treatments. Each value represents and average of two samples taken from each coral (n=3). Bars represent SEM.

margin of difference between these coral and UVT and UVT/UVO acclimatized coral was much less. Coles & Jokiel (1990) also observed a lower photosynthetic rate on both non-acclimatized and 2-month acclimatized coral. Since *F. scutaria* is normally found in shallow (<10 feet deep) water, it may respond more to changes in temperature rather than different levels of UV.

P/R ratios decreased mainly due to the reduction in photosynthetic rates. It is interesting to note that Coles & Jokiel (1990) concluded that higher respiration, and not changes in photosynthesis, is responsible for coral bleaching at elevated temperatures due to both the temperature dependence of biochemical reactions and the inability of coral to maintain high P/R ratios at these temperatures. Respiration at 29°C was not significantly different at any of the three treatments. In addition, coral bleaching was not observed at any time during the experiment. Although a decrease in photosynthesis may consequently lead to reduced growth rates and reproductive capabilities, it may not indeed be responsible for bleaching.

Based upon the present results, UV does not appear to make *F. scutaria* more susceptible to temperature increases. This may be due in part to the lifestyle of this particular coral. Since it is normally found at shallow depths, it must be adapted to its higher UV environment. In addition, it is possible that these corals, which produce a great deal of mucus, may also contain UV-absorbing compounds in their mucus as has been found in *Fungia fungites* (Drollet *et al.*, 1993). In order to fully understand this coral, further measurements of photosynthesis and respiration must be recorded at normal and elevated temperatures to verify these current findings. Mucus and coral tissue samples should also be analyzed for the presence of any mycosporine-like UV-absorbing compounds.

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Preliminary report on the occurrence of mycosporine-like amino acids in the eggs of the Hawaiian scleractinian corals *Montipora verrucosa* and *Fungia scutaria*

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ABSTRACT: The mycosporine-like amino acids (MAAs) of the eggs of the colonial hermaphroditic coral *Montipora verrucosa* and the solitary gonochoric coral *Fungia scutaria* were assayed. The eggs from both species contained relatively high concentrations of MAAs, but differed from each other in quality and quantity of MAAs. These difference may be correlated with differences in their patterns of spawning.

INTRODUCTION

During the past decade, much has been learned about the sexual reproduction of tropical scleractinian corals. Details regarding the modes and timing of some 200+ species are now available (Harrison & Wallace, 1990; Richmond & Hunter, 1990). Old dogmas describing most scleractinians as brooders that release planula larvae (e.g., Hyman, 1940; Vaughn & Wells, 1943) have been rejected after many observations have shown most corals to be hermaphroditic broadcast spawners (reviewed by Fadlallah, 1983; Richmond & Hunter, 1990; Harrison & Wallace, 1990). Many of these broadcasting corals release their gametes synchronously during annual multispecies spawning events (Harrison *et al.*, 1984; Babcock *et al.*, 1986). These mass spawning events are fantastic spectacles of millions of egg bundles drifting to the surface after emerging from their parent polyps.

Yet despite the tremendous amount of information collected during the last decade, no generalizations concerning the various modes of sexual reproduction have been confirmed by observation and experimental study. Thus the adaptive significance of brooding-versus-broadcasting, hermaphroditism-versus-gonochorism, day-versus-night spawning, symbiotic-versus-asymbiotic (possessing or lacking zooxanthellae) eggs and floating-versus-sinking eggs in the life histories of stony corals remain enigmas.

Most broadcasting corals spawn at night when released gametes are not exposed to intense solar irradiation (Harrison & Wallace, 1990). Thus for most coral species, exposure of gametes to damaging ultraviolet radiation may not occur. However, since most of these species produce positively buoyant gametes whose early embryonic stages also float, these embryonic stages may be exposed to full solar radiation during the day following spawning. Consequently, positively buoyant eggs, or the floating embryonic stages, may be expected to possess the ultraviolet radiation blocking substances found in adult corals (Shibata, 1969; Maragos, 1972; Jokiel & York, 1982; Dunlap *et al.*, 1986; Dunlap & Chalker, 1986; Chalker *et al.*, 1988), especially those coral eggs and embryos possessing endosymbiotic algae, which are sensitive to ultraviolet light (Jokiel & York, 1982).

The Hawaiian corals, *Montipora verrucosa* and *Fungia scutaria*, are broadcast spawners that exhibit different patterns of spawning behavior. *Montipora verrucosa*, a colonial hermaphroditic coral, releases positively buoyant egg-sperm bundles between 2030-2230 hours several nights following the new moons of June, July and August (Heyward, 1986; Hunter, 1989). These bundles break apart at the surface where fertilization takes place. The eggs possess endosymbiotic zooxanthellae. By the next morning flattened blastulae may be found floating at the surface of the water.

Fungia scutaria, a solitary gonochoric coral, expels clouds of slightly negatively buoyant eggs at about 1700-1900 hours several nights following the full moons of June through September or October (Krupp, 1983). Note that at this time of day, the sun is low enough on the horizon to be partially blocked by the Koolau Mountains that rise to the west above Kane'ohe Bay, O'ahu. Thus these eggs are spawned at a time when they are not exposed to full solar radiation. *Fungia* eggs, which are much smaller than the eggs of *Montipora* , lack zooxanthellae.

At least some of the differences in spawning behavior among these corals -- floating-versus-sinking eggs and symbiotic-versus-asymbiotic eggs -- may be related to the ability of the eggs and developing embryos to tolerate exposure to solar radiation, especially the damaging ultraviolet rays. As suggested above, eggs that float, yielding floating embryos that are exposed to solar radiation, must possess adaptations (e.g., such as UV-blocking compounds) to minimize the damage suffered from ultraviolet light exposure, especially if these eggs possess UV-sensitive zooxanthellae (Jokiel & York, 1982). Conversely, negatively buoyant eggs that lack zooxanthellae and yield negatively buoyant embryos, might be expected to lack these adaptations. In these respects, the eggs and early embryos of *M. verrucosa* should possess high concentrations of UV-blocking compounds, while those of *F. scutaria* should not.

MATERIALS AND METHODS

Collection and Preparation of Gametes:

Colonies (approx. 20 - 40 cm diameter) of *Montipora verrucosa* were collected from shallow water (less than two meters depth) on the reef surrounding Moku O Loe (Coconut Island) where the Hawai'i Institute of Marine Biology is located. These colonies were either placed in large microcosm tanks (1 m x 1 m x 0.5 m, W x L x D) or individual aquaria receiving continuous seawater one day before the new moons in July and August 1994. At about 1800 h each night, up to four nights following the new moon, the water supply to each tank was turned off. During spawning, floating egg-sperm packets were collected from separate colonies using a filter made from Nitex® plankton netting (120 um) mounted to a two inch segment of PVC pipe (seven inch diameter). Packets collected this way were transferred to a similar net filter placed in a nine inch glass culture dish containing filtered (0.22 um) seawater. Those net filters containing egg-sperm packets were transferred through three changes of filtered seawater.

To break apart the packets, the net filters in the culture dishes were periodically gently agitated. After the packets broke apart, liberating sperm and individual eggs, the eggs were separated from the surrounding sperm suspension by lifting the net filter from the culture dish. This procedure left the sperm suspension behind in the culture dish, while the eggs were retained by the net filter. The net filters containing the eggs were rinsed in filtered seawater three times before preparing for mycosporine-like amino acids (MAAs) extraction and analysis.

Individual specimens of *Fungia scutaria* were placed into glass culture dishes (nine inch diameter) in a large shallow tank during the day of the full moon night in July 1994. These corals had been maintained for more than five months in shallow (10 cm deep) outdoor aquaria receiving a continuous flow of ambient seawater under neutral density shadecloth (about 80% transmittance). Prior to spawning, the tank's water supply was routed through a cartridge filter to remove suspended particulate matter. At about 1530 h, the water level in the tank was lowered to isolate individual corals in their respective culture dishes. The spawned eggs were siphoned from each dish and separately filtered as described above for *M. verrucosa*.

Mycosporine-like Amino Acids Extraction and Analysis:

Aliquots of the fresh egg suspensions were filtered through glass fiber filters (Whatman GF/C) and extracted in methanol (Fisher HPLC grade). These extractions were stored at -50° C until HPLC analysis, the procedure for which is described elsewhere in this volume (Gulko *et al.*, 1995).

Methanol-soluble proteins in the extract were assayed by aliquoting samples into glass tubes and evaporating the solvent before bringing the final volume to one mL with distilled water. These samples were assayed for protein using the Lowry procedure with a BSA standard (Hartree, 1972).

RESULTS

The eggs of *Montipora verrucosa* and *Fungia scutaria* exhibited marked differences in their respective HPLC chromatograms of mycosporine-like amino acids (Fig. 1). The chromatograms for *M. verrucosa* exhibited two prominent peaks corresponding to shinorine and palythine standards. A third major peak did not co-elute with any of the standards and remains unknown. Several minor peaks, possibly corresponding to mycosporine-glycine, porphyra-334 and palythinol, were also apparent on some of the chromatograms.

The chromatograms for *F. scutaria* exhibited a single major peak corresponding to the mycosporine-glycine standard. Several minor peaks (not identified) were apparent on a few of the chromatograms.

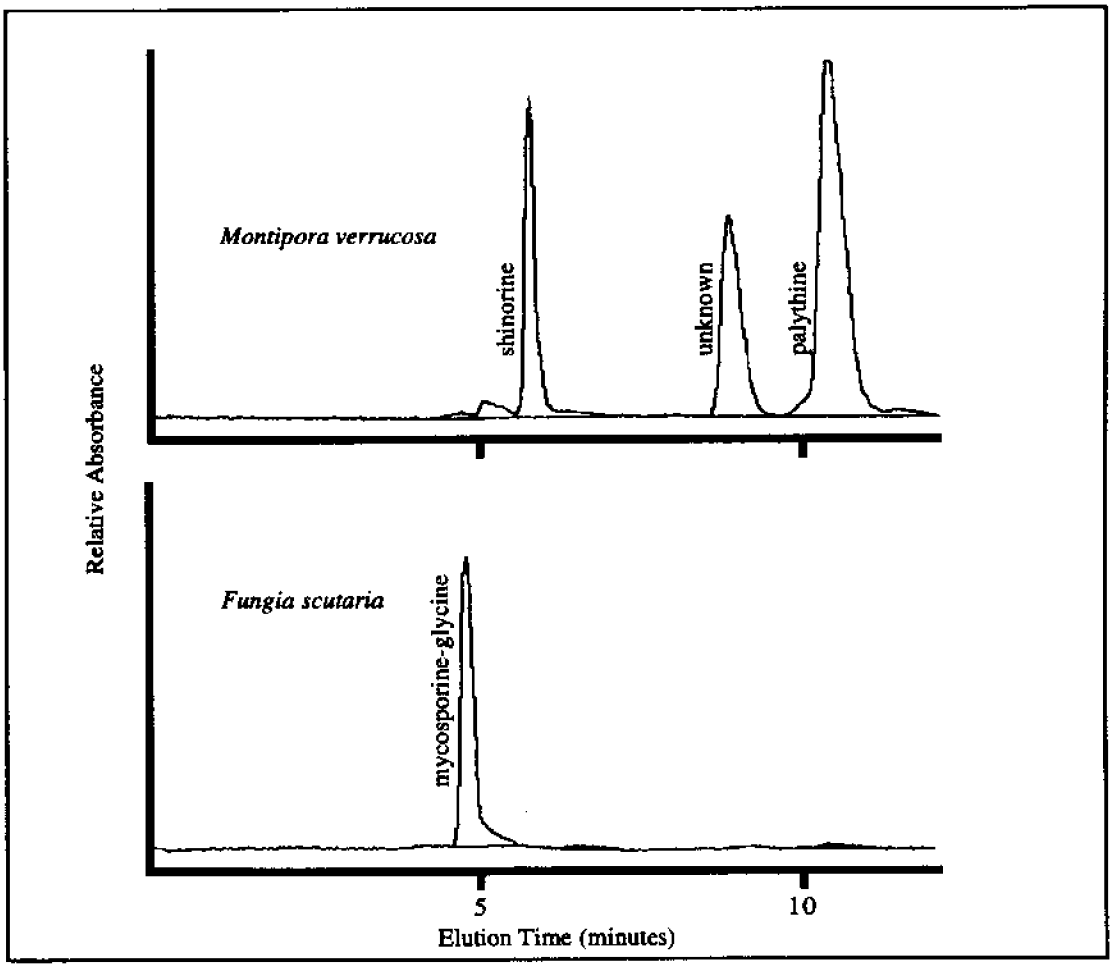

Figure 1. Typical HPLC chromatograms of mycosporine-like amino acids extracted from the eggs of *Montipora verrucosa* and *Fungia scutaria*.

The shinorine content of *M. verrucosa* eggs was estimated at 530 nmol mg^{-1} protein (methanol-soluble protein), while the palythine content was estimated at 837 nmol mg^{-1} protein (Table I). The unknown UV-absorbing eluate could not be quantified, but, judging by its peak height and width, is probably comparable to shirorine. The mycosporine-glycine content of *F. scutaria* eggs was variable, averaging 156 nmol mg^{-1} protein.

Table I. Identified Mycosporine-like Amino Acids in Coral Eggs

	n	nmol/mg protein average	s.d.
Fungia scutaria			
mycosporine-glycine	8	156	74
Montipora verrucosa			
shinorine	3	530	96
palythine	3	837	143

DISCUSSION

The eggs of *Fungia scutaria* and *Montipora verrucosa* exhibited marked differences in the kinds and quantities of their mycosporine-like amino acids (MAAs). The eggs of *Montipora verrucosa*, which float and possess zooxanthellae, contained perhaps 10 - 20x the concentration of MAAs found in the eggs of *Fungia scutaria*, which are negatively buoyant and lack zooxanthellae. These values do fall within the high side of the range of values for MAAs observed in the adult tissues of other shallow water corals (Dunlap & Chalker, 1986; Jokiel *et al.*, MS; Kuffner *et al.*, 1995), holothuroids (Shick *et al.*, 1992), and seaweeds (Banaszak & Lesser, 1995).

Interestingly, the palythine content of the eggs of *Montipora verrucosa* was about 7x the concentration found in adult corals living in shallow water (Kuffner *et al.*, 1995). These shallow water adult corals also lacked substantial quantities of shinorine and an unknown MAA found in the eggs (Kuffner *et al.*, 1995). It appears as though these MAAs are selectively concentrated into the eggs of *Montipora verrucosa*.

The lower MAA content of *Fungia* eggs, when compared to those of *Montipora*, may reflect the absence of zooxanthellae in *Fungia* eggs. Because the only known origin of MAAs is the Shikimate pathway, a complex metabolic pathway characteristic of photosynthetic microorganisms and higher plants (Bentley, 1990), the MAAs in the eggs are assumed to be derived from zooxanthellae, or possibly from foods ingested by the adult corals. This assumption requires that the eggs of *Fungia* acquire their MAAs during gametogenesis from the adult coral. In contrast, the eggs of *Montipora* may acquire some or all of their MAAs from their own zooxanthellae.

The high levels of MAAs found in the floating eggs of *Montipora* may protect the developing embryos and planktonic larval stages from damaging UV radiation. However, *Fungia* eggs, being negatively buoyant, would have a lower need for UV protection. The observed differences in egg MAAs of these two species may thus reflect adaptational differences relevant to their modes of reproduction.

High levels of mycosporine-like amino acids (MAAs) have been reported to occur in the eggs of the scleractinians *Acropora millepora*, *Favia pallida* and *Goniastrea favulus* (Jackson & Babcock, unpublished data, cited by Harrison & Wallace, 1990). It would be interesting to discover if the MAA content of these corals could be correlated with zooxanthellae presence/absence or floating/sinking eggs in these species. Unfortunately, no details regarding MAAs in the eggs of these corals have been reported.

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Effects of ultraviolet radiation on fertilization and production of planula larvae in the Hawaiian coral *Fungia scutaria*

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ABSTRACT: Solar irradiation, specifically within the ultraviolet (UV) range, has been implicated in a variety of effects on corals, but little work has been done on its effects on coral gametes or the resulting planula larvae. Gametes and planula larvae produced by the evening-spawning Hawaiian coral *Fungia scutaria* were exposed to levels of artificial irradiation comparable to daily solar values. Viable planulae production was compared among different irradiation treatments (Full solar (UV-B, UV-A, & PAR), UV-A & PAR, PAR only (no UV), and Dark (no solar irradiation)). Effect of UV exposure was more pronounced on the *F. scutaria* sperm than it was on the eggs or planulae. A strong indication of UV damage was the significant decrease of viable planulae resulting from fertilization with UV-B-exposed sperm. Previous ideas regarding the evolution of nocturnal spawning in corals must be re-evaluated in light of these results.

INTRODUCTION

Ever since Jokiel (1980) first broached the subject of ultraviolet radiation impacting shallow tropical marine organisms, researchers have concentrated on a variety of shallow reef corals (see partial review in Falkowski *et al.*, 1990). Often this work has focused on sessile adult colonies (Dubinsky *et* al., 1984; Gleason, 1993; Jokiel & York, 1982; Vareschi & Fricke, 1986), or the defenses that these benthic organisms utilize to ameliorate the effects of UV (Chalker & Dunlap, 1986; Chalker *et* al., 1988; Dunlap & Chalker, 1986; Dunlap *et al.*, 1986; Drollet *et al.*, 1993; Gleason, 1993). The recent interest in coral bleaching has also led to a number of studies on UV effects (Gleason & Wellington, 1993; Glynn, 1993; Goodman, 1991; Lesser *et al.*, 1990).

Few studies have examined the effects of UV on coral larvae (but see Jokiel, 1985a; and Gleason & Wellington, 1995). While studies on sessile corals are important, the adult coral colonies are exposed to less UV (though over a much longer and extended period) and often have well-established protective mechanisms (mycosporine-like amino acids (MAAs), pigments, shade adaptation, etc.), than the gametes or planulae in the water column above the reef, depending on the time of gamete release. Gleason & Wellington (1995) looked at the effect of UV on *Agaricia agaricites* planulae and found a decrease in survival of planula larvae released from adult colonies at different depths. They related contrasts in planula survival to differences in the concentrations of MAAs incorporated into the planulae released from different depths. *Agaricia* broods its larvae prior to release and produces large planulae containing zooxanthellae. Studies done on *Pocillopora damicornis*, another brooding species in Hawai'i that produces planulae containing zooxanthellae, have shown the ability of these larvae to remain planktonic for over two months (Richmond, 1982, 1987). Such a situation would place the larvae under extensive exposure to high levels of UV irradiation. Presumably, zooxanthellae, whose pigments and postulated production of MAAs through the Shikimate pathway (Bentley, 1990) provide shielding from the effects of UV, are incorporated into the planulae prior to release from the adult colony. Similarly, lipids or pigmented bodies stored in the planulae could serve to also help protect structures such as DNA from solar irradiance.

The majority of coral species so far observed appear to broadcast spawn their gametes as opposed to brooding planulae (Kojis & Quinn, 1982; Richmond & Hunter, 1990; Harrison & Wallace, 1990). Sperm and eggs released into the water column, and the externally-formed planula produced from them, may lack some (or all) of the defenses against UV seen in brooded planula.

Since eggs are more dense then sperm, often pigmented, and considering that many coral eggs have zooxanthellae already in them (and therefore presumably a source of MAA compounds), one would expect the detrimental effects of UV on eggs to be less-pronounced than for sperm which lack such possible defenses. While not expecting large-scale mortality of sperm, surface UV might result in a decrease of movement or ability to penetrate the egg. Likewise, UV might cause changes in the permeability of the egg.

The present investigation evaluated the effects of UV on *Fungia scutaria* coral sperm and the ability of irradiated sperm to successfully fertilize *F. scuteria* eggs, the effect of UV independently on *Fungia* eggs, and the effect of UV on planula larvae produced from non-irradiated eggs and sperm.

MATERIALS & METHODS

Solitary *Fungia scutaria* were collected from various patch reefs in Kane'ohe Bay in order to maximize genetic diversity among individuals and minimize self-fertilization or non-fertilization due to relatedness. Corals were carefully and quickly transported to the Hawai'i Institute of Marine Biology where they were maintained separately in glass bowls set within flow-through wet tables. Prior to a spawning event, the wet tables were cleaned and the water changed to cartridge-filtered (0.22 µm) flow-through seawater. The water level was lowered prior to the onset of a spawning event, resulting in each coral's spawned gametes being retained within that individual's glass bowl for identification and collection.

Gametes were collected from individually spawning corals during the annual summer spawning periods (June through September, two to four nights after the full moon, between 1700 and 1900 hours). Gametes were immediately collected after spawning by gently siphoning the isolated water mass within a spawned coral's glass bowl.

Sperm Exposure Experiment

Sperm from three spawned males were gently mixed together in a 500 ml beaker in order to minimize individual genetic problems with sperm that might affect fertilization success. Five ml of this sperm mixture was then gently poured into each of twelve, clean, foil-covered borosilicate 30 ml shell vials containing 20 ml of 0.22 µm filtered seawater .

Four treatments were prepared:

- a UV transparent (UVT) treatment that allowed PAR + UV-A + UV-B radiation to pass through a lid fitted with Aclar® 33c Fluoropolymer film (produced by Allied Signal Plastics (Pottsville, PA), 127 µm thickness (5 gauge)).
- a UV-A transparent (UVA) treatment that allowed PAR + UV-A radiation to pass through a lid fitted with Mylar® Type D Fluoropolymer film (produced by DuPont, 127 µm thickness (5 mil)).
- a UV opaque (UVO) treatment that blocked both UV-A and UV-B, allowing only PAR radiation to pass through a lid fitted with 100% Clear Acrylic Safety Glazing sheet produced by K-S-H, Inc., 2.5 mm thickness.
- a dark (Dark) treatment that prevented irradiation of the sample, but that was within the same water bath as the other treatments.

Data on the optical properties of the filters used in this experiment is contained in Gulko *et al.* (this volume).

Each of the twelve shell vials was randomly assigned and fitted with a fliter lid resulting in three replicates per treatment (n = 3). Each of the filtered shell vials were then placed randomly underneath the center of a light field generated by a water-cooled, AIRMASS 1 filtered KRATOS Solar Simulator (Solar Simulator) and exposed to solar spectral output similar to that of natural sunlight measured beneath the surface in Kane'ohe Bay. Figure 1 shows a comparison of spectral irradiance directly adjacent to HIMB in Kane'ohe Bay with that of the HIMB Solar Simulator at the setting (75 amps) used throughout this experiment. This setting resulted in spectral irradiance similar to that seen in Kane'ohe Bay at a depth of 3.5 m. Under natural conditions, sperm, eggs or planulae floating near the surface would be exposed to higher mid-day levels of UV than used in this set of experiments. Treatments were exposed to simulated sunlight (UV plus PAR) from the Solar Simulator for 60 minutes. The dark treatments were maintained in the same water bath (27°C) but outside of and shielded from the irradiation field produced by the Solar Simulator.

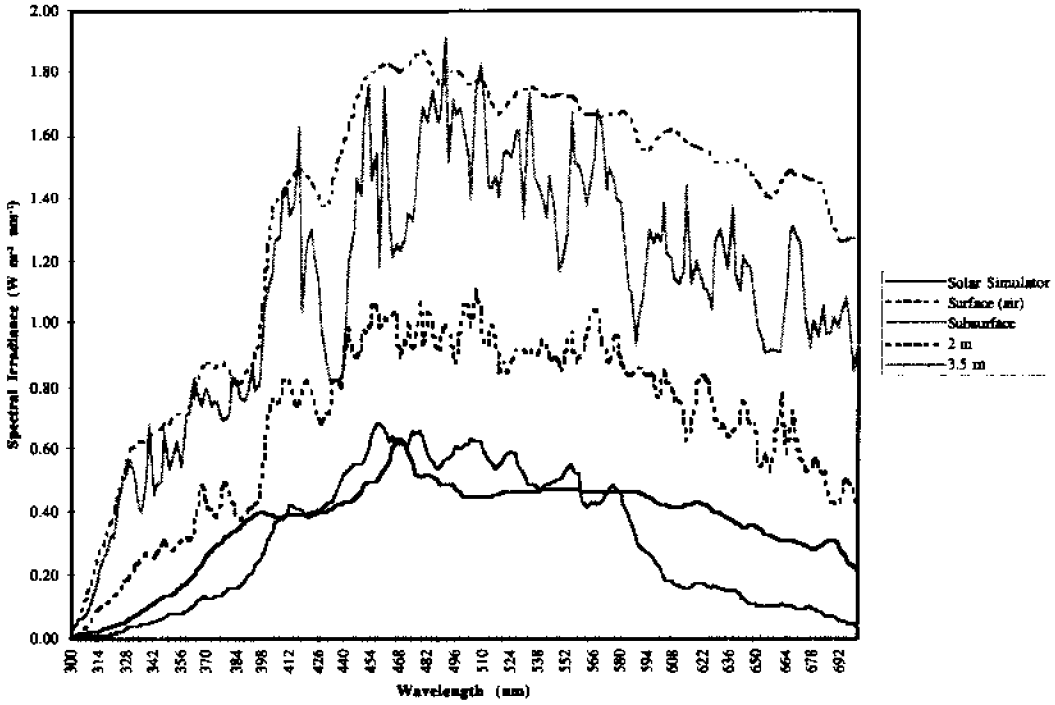

Figure 1. Comparison of spectral irradiance scan of the HIMB Solar Simulator with spectral irradiance scans taken at different depths immediately adjacent to the Hawai'i Institute of Marine Biology in Kane'ohe Bay. Taken under clear skies on July 26, 1994, between 12:05 pm and 12:30 pm.

While the sperm treatments were being irradiated, an unexposed 10 ml sample of diluted sperm from the 500 ml source beaker was subsampled to determine sperm densities using a hemocytometer and then scored for sperm motility. Also during this time, freshly spawned *Fungia scutaria* eggs were gently pipetted into clean 30 ml shell vials containing 20 ml of 0.22 µm filtered seawater. Each vial was numbered and the number of eggs contained within was recorded; within each vial, each egg represented a potential planula larva. Any irregular or "blown-out" eggs were discarded prior to counting.

After exposure, 1 ml of sperm from each vial was gently pipetted into a 30 ml shell vial containing the *Fungia* eggs (between 40 - 100 eggs) and filtered seawater. After ten minutes of gentle agitation to provide for maximum fertilization, the egg-sperm mixture was transferred to clean 250 ml jars containing 150 ml of 0.22 µm filtered seawater at 27°C. The jars were then tightly capped and placed within a shaded water table supplied with flow-through seawater overnight. This arrangement allowed gentle agitation of the fertilized eggs at ambient temperature. Except for exposure treatment of sperm to UV, all handling and incubation of gametes was conducted under low-light conditions.

Sperm samples from each treatment were sampled for motility and compared with the motility of an untreated sperm sample from the original sperm mixture.

Twelve hours after fertilization, each sample was censused for the number of planulae or pre-planulae (developing eggs) present. The mean percentage of planulae and pre-planulae was then compared among treatments. Results were analyzed by arcsine transformation of percentage survival data, and single-factor ANOVA tables were generated using a Microsoft Excel Statistical Analysis Toolpak.

Egg Exposure Experiment

Eggs and sperm from recently spawned *Fungia scutaria* individuals were collected as described above. Eggs were pipetted into each of nine, clean, foil-covered borosilicate 30 ml shell vials containing 20 ml of 0.22 µm filtered seawater ; the shell vials were fitted with lids as described

above for the sperm experiment. Unlike the sperm and planula experiments, a dark treatment was omitted for this experiment due to the need to minimize time between UV exposure of eggs and fertilization, and the need to count eggs under a microscope with a strong light field. This resulted in a total of nine shell vials, comprising three treatments, which were arranged randomly underneath the center of a light field generated by the Solar Simulator and exposed to solar spectral output similar to that of natural sunlight measured in Kane'ohe Bay as described for the sperm exposure experiment. Treatments were exposed to simulated sunlight (UV + PAR) from the Solar Simulator for 60 minutes.

While the egg treatments were being irradiated, freshly spawned *Fungia scutaria* sperm were collected and censused for density and motility.

After exposure, 1ml of sperm was gently poured into each 30ml shell vial containing the exposed *Fungia* eggs. After ten minutes of gentle agitation to provide for maximum fertilization, the eggs in each egg-sperm mixture were counted and then transferred to clean 250 ml jars containing 150 ml of 0.22 µm filtered seawater at 27°C. The jars were then tightly capped and placed within a shaded water table supplied with flow-through seawater overnight. Except for exposure treatment of eggs to UV, all handling and incubation of gametes was conducted under low-light conditions. After twelve hours, each sample was then censused and analyzed as described above for the sperm exposure experiment.

Planula Larvae Exposure Experiment

Eggs and sperm were collected as described above from recently spawned *Fungia scutaria* individuals on 10 September 1995 and allowed to fertilize. The resulting fertilized eggs were allowed to develop into planula larvae within 3-gallon aquaria containing 0.22 µm filtered seawater. After 24 hours, planulae were counted and pipetted into each of twelve, clean, foil-covered borosilicate 30 ml shell vials containing 20 ml of 0.22 µm filtered seawater; the shell vials were fitted with filter-lids as described above for the sperm experiment. The twelve shell vials of planulae (n = 3 per treatment) were arranged randomly underneath the center of a light field generated by the Solar Simulator and exposed to spectral irradiance similar to that of natural sunlight measured in Kane'ohe Bay as described for the sperm exposure experiment. Treatments were exposed to simulated sunlight (UV plus PAR) from the Solar Simulator for 60 minutes.

After exposure, the planulae were transferred to clean 250 ml jars containing 150 ml of filtered seawater (at 27°C). The jars were then tightly capped and placed within a shaded water table supplied with flow-through seawater. Except for exposure treatment of planula larvae to UV, all handling and incubation of planulae was conducted under low-light conditions. After twelve hours, each sample was then censused and analyzed as described above for the sperm exposure experiment.

RESULTS

In general, sperm counts within any one set of experiments did not vary notably between treatments as measured through counts using a hemocytometer (pers. obs.).

Sperm Exposure Experiment

Survival of *F. scutaria* planulae twelve hours after fertilization was dramatically decreased for planula larvae produced from sperm irradiated under UVT conditions than that seen with the other three treatments (Fig. 2). Table I shows this difference to be highly significant ($F = 17.382$, $P < 0.01$). While sperm counts in this experiment did not noticeably differ, sperm motility did vary between treatments (unreported data, manuscript in preparation) and appears to be strongly affected by UVT.

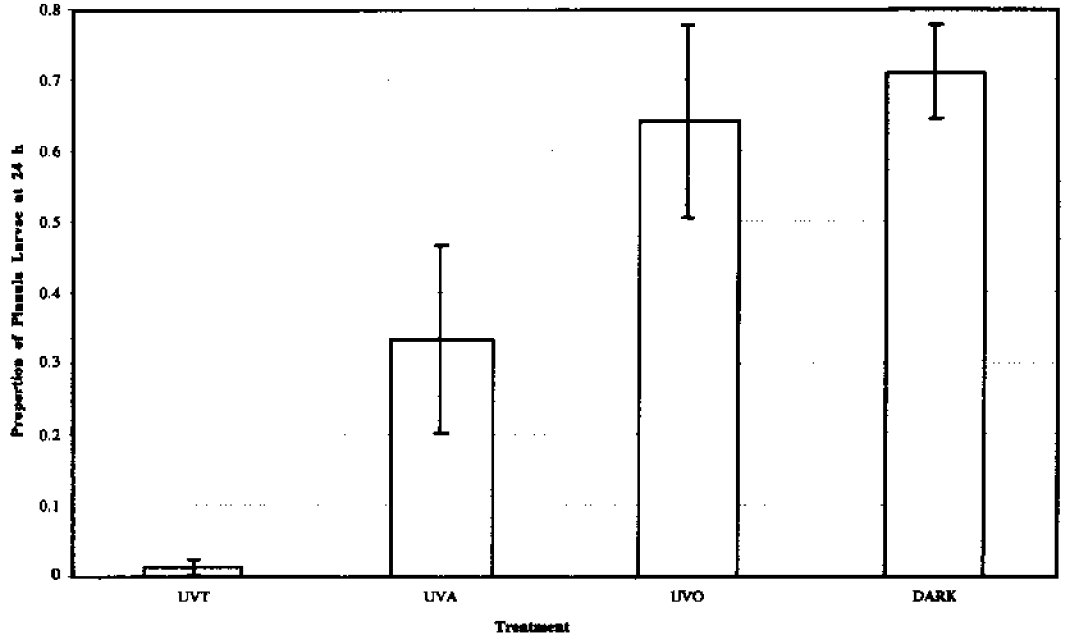

Figure 2. Proportion of the potential number of *Fungia scutaria* planula larvae (based on initial egg count) in each treatment at 12 hours after sperm exposure.

Table I. ANOVA table of results of *Fungia scutaria* sperm exposure treatments.

Source of Variation	df	SS	MS	F	P-value
Between Treatments	3	1.171	0.3905	17.382	0.0007
Within Treatments	8	0.1797	0.0225		
Total	11	1.3512			

Egg Exposure Experiment

Planula larvae produced from eggs exposed to varying UV treatments did not appear to differ significantly in their rate of survival twelve hours after fertilization (Fig. 3; Table II). Differences in percentage larval survival between this experiment and the sperm (or planula larvae) experiment are not comparable due to differences in spawning periods, times and experimental set-up.

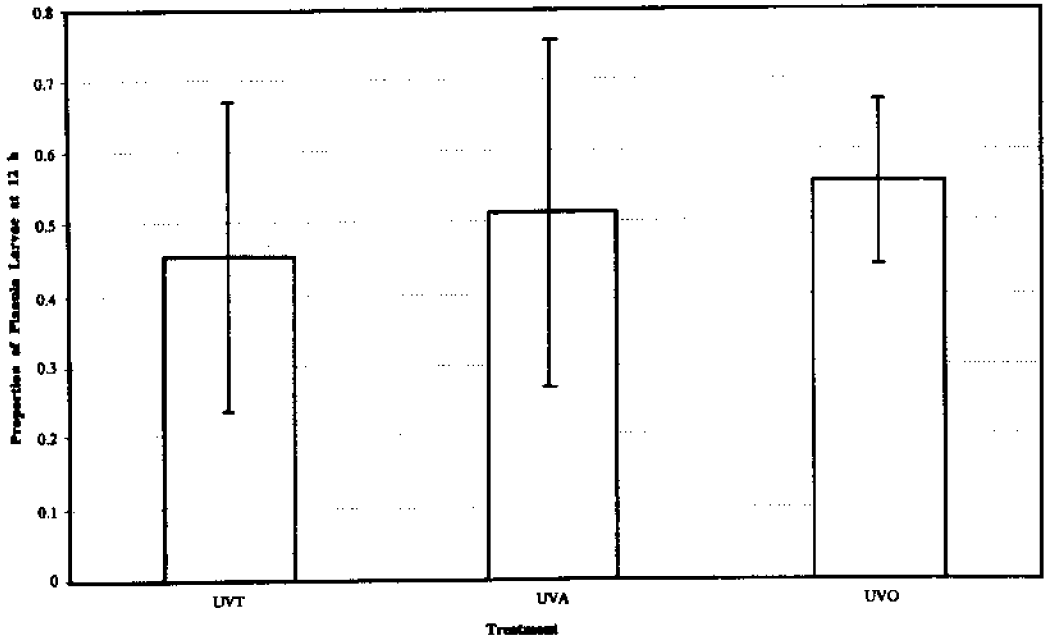

Figure 3. Proportion of the potential number of *Fungia scutaria* planula larvae (based on initial egg count) in each treatment at 12 hours after egg exposure.

Table II. ANOVA table of results of *Fungia scutaria* egg exposure treatments.

Source of Variation	df	SS	MS	F	P-value
Between Treatments	2	0.0174	0.0087	0.0996	0.9066
Within Treatments	6	0.5251	0.0875		
Total	8	0.5425			

Planula Larvae Exposure Experiment

The number of planula larvae surviving twelve hours after exposure did not vary between UV and PAR treatments (Fig. 4; Table III). A difference was noted between UV treatments and the Dark treatment, although the Dark treatment did not significantly differ from the UV-opaque (PAR) treatment. This suggests a UV effect, but this result was not as pronounced as that seen for the sperm experiment.

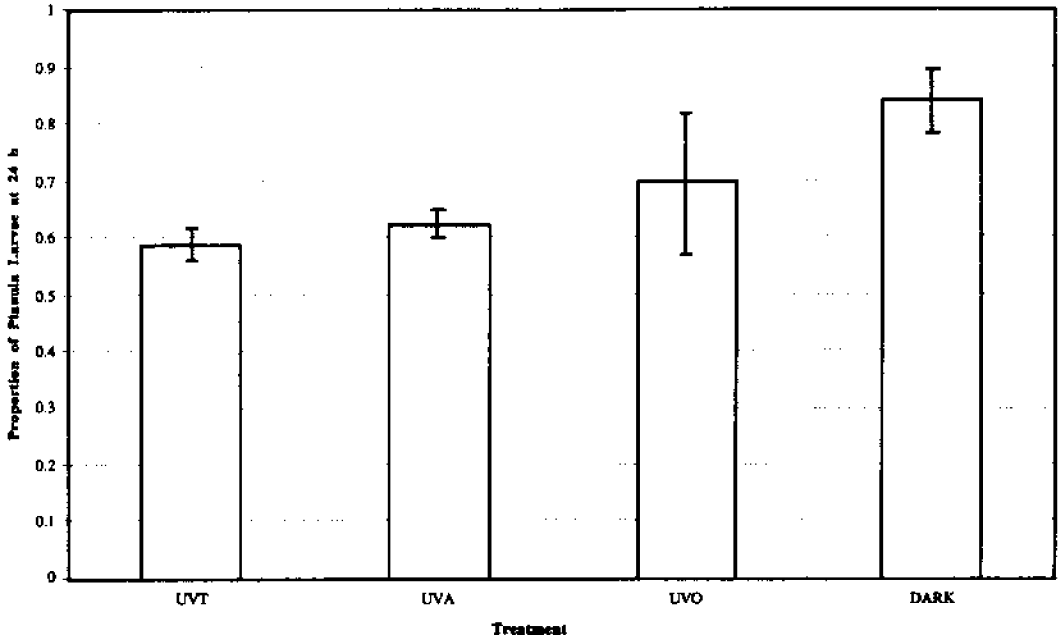

Figure 4. Proportion of the potential number of *Fungia scutaria* planula larvae (based on initial planulae count) in each treatment at 12 hours after planulae exposure.

Table III. ANOVA table of results of *Fungia scutaria* planula larvae exposure treatments.

Source of Variation	df	SS	MS	F	P-value
Between Treatments	3	0.2541	0.0847	4.522	0.039
Within Treatments	8	0.1499	0.0187		
Total	11	0.4040			

DISCUSSION

The results of this study clearly demonstrate the importance of ultraviolet radiation on the production of viable larvae in broadcast spawning species whose sperm might be exposed to such conditions. Additionally, *Fungia* planula larvae exposed in the current experiment also showed some effects on survival due to UV. Though lacking zooxanthellae, *Fungia* planulae do contain lipids and MAAs, as the eggs they were produced from contain measurable amounts of MAAs (Krupp & Blanck, this volume). Presumably, these provide some protection against UV exposure; though the concentration in the eggs may be greatly diluted in the developed, much larger planula larvae without an influx or production of new MAAs. If this were the case, it might account for the decreased survival of planula under UV conditions which a was not observed in the irradiated egg experiment. In the wild, *Fungia* planulae resulting from negatively buoyant eggs would not be swimming high in the water column and exposed to such UV levels. Additionally, *Fungia* larvae are thought to acquire zooxanthellae either while in the plankton or shortly after settlement, and would then have both a source of pigments and MAAs to enhance their own protection. Given the presence of concentrated MAAs in the *Fungia* eggs, it not

surprising that larvae produced from UV-exposed eggs in the present experiments did not show significant decreases in percentage survival. A recent study by Krupp and Blanck (this volume) compared specific MAA content of *Montipora verrucosa* eggs with data on MAA content in the adult colony at shallow depth. They found the eggs to have seven times the amount of one type of MAA concentrated within them, suggesting that MAAs are selectively concentrated in the eggs to protect these gametes (and the developing planula larvae) while in the water column. It should be noted that *Montipora* concentrates zooxanthellae into the eggs prior to spawning and this could account for the high presence of MAAs within the eggs. Studies done in Okinawa (Heyward *et al.*, 1987) suggest that few other species implant zooxanthellae within their eggs prior to spawning; as such, the presence of MAAs in non-zooxanthellae eggs represents yet another energetic expense on the part of the adult.

Even though UV-B makes up less than 0.3% of the solar irradiance reaching the surface of the ocean, there is enough ambient UV-B present to damage poorly-shielded cellular DNA (Kohen *et al.*, 1995). A number of studies have been done on the effects of UV on the gametes of aquatic organisms, but the majority of these have been associated with vertebrates such as frogs and fish (Grunwald & Streisinger, 1992; Dey & Damkaer, 1990; Ijiri, 1980a, 1980b). Most of the studies have shown a sterilizing effect on the eggs given high doses immediately after fertilization. When sperm were irradiated at low doses and then used to fertilize normal eggs, survival was very low, while highly irradiated sperm resulted in a higher proportion of surviving offspring. This phenomena is referred to as the 'Hertwig Effect' and is thought to result from the sperms' chromatin being totally inactivated causing the larvae to be produced by the maternal haploid set of chromosomes (Ijiri, 1980a, 1980b). Aquaculture workers have used UV light to purposely inactivate sperm to induce gynogenesis in active eggs in order to produce monosex generations (see Goryczko *et al.*, 1991).

Unlike planulae or eggs, sperm are relatively poorly protected from the effects of UV and react strongly to UV exposure. *Fungia scutaria* sperm contain little, if any, MAAs (D.A. Krupp, pers. comm.) and showed significant effects from exposure to UV-B in this study. Given the comparable size and energetic investment differences between individual sperm and eggs, this is perhaps not surprising.

The results do not clearly indicate whether UV-B prevented sperm from successfully fertilizing eggs, or allowed sperm to fertilize the eggs but not successfully develop into planula larvae due to mutation of the DNA (or possibly a combination of these two effects). UV-B causes malformations in *Montipora verrucosa* planulae produced from UV-B irradiated sperm under conditions similar to those described for this experiment (Gulko, unpublished observations), suggesting mutation of the DNA without complete de-activation of fertilization capability.

Two specific types of sperm have been identified in scleractinian corals (Harrison, 1985a; Harrison & Wallace, 1990). An ovoid- or pear-shaped sperm is generally characteristic of hermaphroditic species, while sperm with an elongated head containing a conical structure at the apex is more characteristic of gonochoric families (including the Fungiids). The generalized structures of these two types of sperm are as shown in Figure 5.

Other possible sites of action on the sperm include specific constituents of the motor apparatus involved with the flagella (such as the mitochondria, which supply the energy for flagellar movement), receptor sites involved in sperm orientation (chemotaxis) towards the egg, or as yet unidentified sites comparable to the enzyme-containing cap (acrosome)[1] seen on the head of other invertebrate sperm and involved in sperm penetration of the egg (Fig. 5). Studies on green flagellates after exposure to UV-B have shown loss of motility (Kohen *et al.*, 1995). Similar results have been reported with filamentous, gliding cyanobacteria (Donkor & Hader, 1991). Both of these studies suggest that UV has effects on sites other than the DNA. For coral sperm, this could strongly affect the ability of the sperm to reach or penetrate the egg.

[1] Coral sperm lack a well-defined acrosome, but contain small vesicles (termed "pro-acrosomal" vesicles) which may have a similiar function (Harrison & Wallace, 1990).

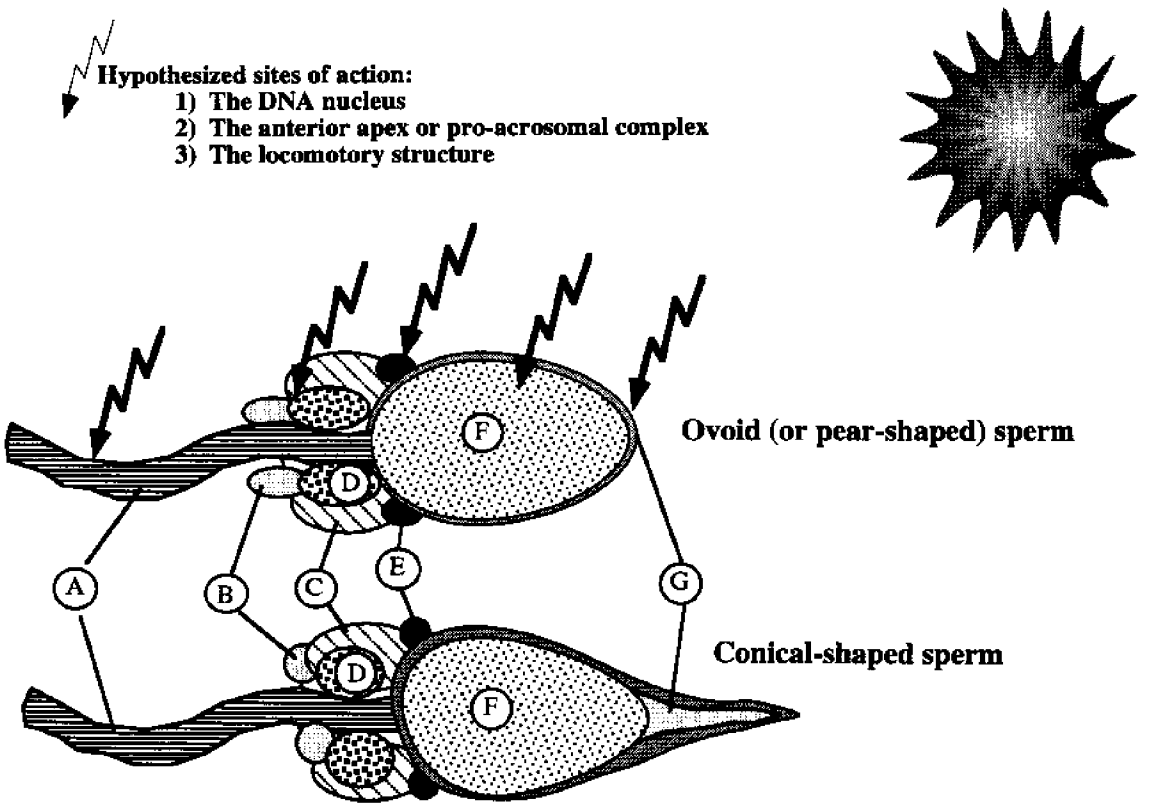

Figure 5. Diagram detailing the two types of sperm described for scleractinian corals (After Harrison, 1988b, as shown in Harrison & Wallace, 1990) along with hypothesized sites of action following exposure to UV-B radiation. A, flagellum; B, cytoplasmic collar; C, lamellae; D, mitochondria; E, pro-acrosomal vesicles; F, DNA-containing nucleus; G, apical less dense nuclear zone.

Sperm behavior prior to fertilization may also be impacted. When coral sperm encounter an egg they aggregate to form "sperm clots" adhered head-first to the egg surface (Babcock & Heyward, 1986); the function of such behavior is still unknown, but may play a role in facilitating fertilization. Inactivation, or modification of this behavior due to UV exposure, could contribute to the decreased fertilization observed (as represented in the current sperm experiments by decreased survival of planula larvae).

Outside of environmental cues such as seasonal temperature change, lunar and tidal cycles, and with very few exceptions [*Fungia concinna, Pavona cactus* (Marshall & Stevenson, 1933), *Fungia scutaria* (Krupp, 1983), *Pocillopora verrucosa, P. eydouxi* (Kinzie, 1993)[ii]], hermatypic corals specifically appear to spawn primarily at night. Among the various cues responsible for spawning, nocturnal illumination has been proposed to coordinate the timing of release (Jokiel *et al.*, 1985; Jokiel, 1985a; Jokiel, 1985b; Richmond & Hunter, 1990).

According to a number of propagule dispersal hypotheses, many marine animals spawn during the late day or early evening to avoid egg predation by diurnal planktivorous fish. Such a strategy has been proposed for both reef fish (Robertson & Hoffman, 1977; Johannes, 1978; Meyers, 1989; Robertson, 1991) and corals (Harrison *et al.*, 1984; Wallace *et al.*, 1986; Babcock *et al.*, 1986; Harrison & Wallace, 1990) with the idea that by restricting spawning to late day/early evening, diurnal egg predators would either be inactive or, if active, satiated from diurnal feeding. While coral spawn has yet to be shown to be a major prey item for planktivorous fish, Westneat and Resing (1988) did find that some fish switched their diet to coral spawn during mass coral spawning events.

[ii] Kinzie reported these species spawning early in the morning in Japan; conversely, Schlesinger & Loya (1985) reported *P. verrucosa* released its gametes at night on reefs in the Red Sea.

Results of this investigation demonstrate that free-spawned gametes, being vulnerable to the effects of UV, have a higher survival/success rate if spawned at night (or late in the afternoon) when UV levels would be very low. Under such a UV-avoidance hypothesis, corals (or any marine organism whose gametes contain little, if any, defensive mechanisms against UV radiation, and whose gametes raise up off the reef and into the surface layers[#]) would have evolved behavioral strategies such as night-time spawning in order to maximize fertilization success. Night-time spawning may have evolved, in part, to allow gametes maximum time for fertilization and development prior to UV exposure, and to minimize exposure of the poorly shielded sperm from high UV in an environment where diurnal broadcast spawning would expose sperm to such conditions. Even those species that do not spawn at night characteristically spawn at times when UV levels are low. The results of this work show a strong UV-B effect on coral sperm which affects their ability to successfully produce planula larvae. Such results argue strongly for a UV-avoidance hypothesis of night-time spawning in corals. Currently, very little evidence exists to support the alternative predation hypothesis for night-time spawning in corals.

Ongoing work is presently looking at the effects of UV on the eggs and sperm of a hermaphroditic coral which releases egg-sperm bundles in the evening hours on the new moon. Preliminary results suggest a similar effect on the sperm as seen with *Fungia*. Other research will explore both the actual effects on the sperm itself, and endeavor to offset *Fungia scutaria* individual's biological clocks in an attempt to induce spawning during mid-day hours so that sperm can be exposed to natural UV conditions.

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[#] Presumably for dispersal, but possibly also to avoid concentrations of egg predators on the reef

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Solar UV-A inhibition of planula larvae in the reef-building coral *Pocillopora damicornis*

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ABSTRACT: Newly-released planulae from adult colonies of the scleractinian coral *Pocillopora damicornis* were subjected for 2 weeks to one of 3 UV treatments using solar filters. In those treatments where UV-A and UV-B were screened out, the rate of larval settlement increased, and the final number of larvae settled (reproductive success) reached 37.3% (±3.7%), compared to 20.6% (±4.9%) with removal of UV-B alone, and 13.1% (±3.1%) under control conditions with neutral density filters. These results argue for the ecological significance of UV, particularly in the UV-A region of the spectrum, as an important factor determining larval recruitment. Acclimation of parent coral colonies to different UV treatments for one month prior to release of planulae did not affect settlement rates or final numbers of larvae settled. HPLC analysis revealed that newly-released planulae contain at least five different types of mycosporine-like amino acids (MAAs), which are polar compounds known to absorb strongly in the UV region. Acclimation of parent colonies to different UV treatments produced planulae which varied in their MAA content. This variation was dependent on both the intensity and spectral composition of the UV treatment, and on the absorbance characteristics of the particular MAA concerned. However, MAA content did not appear to directly affect the settlement success of the planula larvae. Therefore, whilst this study documents variation in MAAs in response to UV radiation, it does not support the hypothesis that MAAs necessarily provide a protective barrier against the effects of high energy radiation.

INTRODUCTION

Solar ultraviolet radiation in the 290 - 400 nm band has only relatively recently been recognized as detrimental to tropical marine shallow-water benthic organisms (Jokiel, 1980; Siebeck, 1981; Worrest, 1982; Siebeck, 1988; Glynn, 1992; Gleason & Wellington 1993). Until the early 1980s, its importance as a physical stressor on coral reefs was underestimated, largely due to (i) a general belief that only modest amounts of UV penetrated to significant depth, and (ii) the observation that UV-A (320 - 400 nm) and UV-B (280 - 320 nm) are together only a fraction (~4 - 7%) of the radiant flux emitted in the visible region of the spectrum. However, data demonstrating significant aquatic UV penetration has been available since at least Jerlov's (1950, 1968) studies, and was more comprehensively expanded over 15 years ago (Smith & Calkins, 1976; Smith & Baker, 1979, 1981). Additionally, the effects of even small amounts of high energy UV radiation on a variety of biochemical and subcellular processes in marine organisms is well documented (Worrest, 1982; Häder & Worrest, 1991). It is somewhat surprising, therefore, that studies have only recently demonstrated the ecological significance of UV in the often clear tropical waters associated with coral reefs (Gleason & Wellington, 1993).

Previous studies of the biological effects of UV have generally emphasized UV-B as the major biocidal component of UV radiation (Worrest, 1982; Smith & Baker, 1989; Häder & Worrest, 1991). However, since UV-A is typically an order of magnitude higher in terms of its contribution to total radiant flux, its detrimental effects may have been underestimated.

Hermatypic coral tissue has been shown to possess compounds (S-320), commonly called mycosporine-like amino acids (MAAs), that absorb strongly in the UV region (Shibata, 1969; Dunlap & Chalker, 1986). The varying abundance of these compounds under different natural conditions of UV irradiance has suggested an adaptive value for these compounds, although the evidence for this so far is only correlative. However, reef-building corals *in situ* are also afforded protection by their own skeletal components and by shading from neighboring reef material. Therefore it would appear likely that detrimental effects of UV on corals are more likely to be apparent when the coral undergoes a planktonic reproductive or dispersal phase in its life cycle, especially if the reproductive propagules are positively buoyant or are swept up to shallower depths, thereby receiving higher doses of UV.

Some authors have suggested that the larval stage may be the more resistant phase in a coral's life cycle (Edmondson, 1946; Coles, 1985; Esquivel, 1986). Whilst this may be true for some aspects of larval survival, particularly considering long-term competency of larvae and low metabolic demands whilst in the plankton, the same may not be true for high UV dosage and dose rate, where the potentially greater susceptibility of the larvae, in part due to surface area to volume considerations, is apparent.

The planula larvae of the hermatypic scleractinian coral *Pocillopora damicornis* are released monthly in Australia, Hawai'i and Micronesia (Marshall & Stephenson, 1933; Harrigan, 1972; Stimson, 1978; Richmond & Jokiel, 1984). Since planula larvae contain zooxanthellae and also

have a high energy value, the potential planktonic lifetime of the larvae is thought to be long (Richmond, 1982, 1987). This long period of larval competency allows for considerable substrate selection by the free-swimming larva.

Larval settlement, defined for *P. damicornis* as the attachment of the planula to the substratum followed immediately by spreading of the thin basal plate, formation of calcareous septa, and the appearance of tentacular buds around the central mouth (Harrigan 1972), is highly dependent on both substrate type and local environmental conditions. These environmental conditions may be biotic or abiotic, and include light (Harrigan, 1972; Lewis, 1974; Birkeland, 1977), sediment (Birkeland, 1977; Rogers *et al.*, 1984; Rogers, 1990; Te, 1992), wave action (Birkeland *et al.*, 1982), grazing (Brock, 1979; Harriott 1985), algal competition (Sammarco, 1982; Rogers *et al.*, 1984) and anthropogenic marine pollutants (Te, 1991).

Settling larvae prefer cryptic microhabitats, away from direct light (Kawaguti 1941), which have been 'conditioned' in some way by an algal or bacterial film, or some chemical stimulus (Morse & Morse, 1992). The choice of cryptic habitats, however, may be influenced by a number of factors. Cryptic habitats with low ambient PAR levels may reduce competition and overgrowth by turf and macroalgae, hence increasing recruitment (Birkeland, 1977). However, higher irradiance in the PAR region of the spectrum should provide direct autotrophic benefits to early larval development (Lewis, 1974). Furthermore, the inaccessibility of cryptic sites to grazing by larger vertebrates and invertebrates, which is only secondarily correlated with lower light levels, may also lead to an increase in initial success (Birkeland & Randall, 1982).

Given that high solar radiant flux has been shown to have an inhibitory effect on initial settlement (Kawaguti, 1941), this study aims to separate the different regions of the solar spectrum to elucidate a broad-band action spectrum for the reduction in settlement under high natural radiation conditions. It is hypothesized that high irradiance in the UV region of the spectrum is a significant determinant of larval substrate selection. When energy resources are limited, or mortality in the planktonic phases is high, variations in time spent selecting a suitable site translates directly into differential reproductive success.

The potential effects of solar UV on the settlement process, and indeed most other aspects of coral reproductive biology, have been largely ignored (but see Jokiel, 1985). This study aims to demonstrate the importance of such effects, and suggests significant gaps in our understanding of environmental factors affecting larval recruitment in scleractinian corals.

MATERIALS AND METHODS

Adult Colony Collection and Acclimation to UV Treatment

Adult colonies of *Pocillopora damicornis*, 10-15cm in diameter, were collected at depths of 1-2 m from patch reef no. 40, Kane'ohe Bay, O'ahu, Hawai'i on 23 June 1994. Colonies were observed for planula release using planulae collectors similar to those described in Richmond (1985). A large number of genetically distinct types of *P. damicornis* occur in Kane'ohe Bay, each planulating on different days of the lunar cycle (Stoddart, 1984; Jokiel, 1985). From those collected colonies which planulated in quantity over a six day period following the full moon of 23 June, 12 were selected and randomly allocated to one of three treatments (4 colonies per treatment):

Table I: Different materials used to block various wavelengths of solar radiation. UVO blocks UV radiation and allows only PAR (>400 nm) through. UVA blocks UV-B radiation and allows wavelengths greater than 350 nm through. UVT allows both UV and PAR through (290 nm and greater). See also Figure 1 and Gulko *et al.* (this volume).

TREATMENT LEVEL	FILTER TYPE *(SEE ALSO FIGURE 1)*	RADIATION PASSED
UVO	Plexiglas® G-UF-3 Acrylic sheet (6.0 mm) *(Polycast)*	PAR only
UVA	Mylar® Type D Fluoropolymer film (127mm) *(DuPont)* + Plexiglas® G-UVT Acrylic sheet (6.0 mm) *(Rohm & Haas)*	PAR + UV-A
UVT	Plexiglas® G-UVT Acrylic sheet (6.0 mm) *(Rohm & Haas)*	PAR + UV-A + UV-B

Each treatment was carried out in one of three 410 liter tanks supplied with flow-through seawater at 8 liters per minute. Corals were left in these treatments throughout a single lunar cycle, from the end of planulation on 29 June until the full moon of 22 July.

Over the lunar cycle, treatments and tanks were rotated twice, at 9-day intervals, to prevent potential confounding tank effects. Each treatment thus spent equal time in each tank.

Planulae released from the corals over the period 21-25 July 1994 were collected. Adult colonies were removed from the acclimation tanks, planulae were collected overnight, and the colonies were returned to the treatment tanks the following morning. On 23 July, sufficient planulae were released over a single night to enable the use of planulae released from adult colonies at the same time. Planulae from the 4 colonies subjected to each treatment were pooled to produce 3 groups of planulae produced from colonies acclimated to different UV regimes (see Figure 2). Planulae released from the corals on other nights were analyzed for MAAs by extraction of 25 - 50 planulae in methanol and analysis by HPLC (see Gulko *et al.*, this volume).

Rate of Planula Settlement under UV Treatment

The planulae obtained from the colonies acclimated under the three different UV treatments were then allocated to settlement containers and re-subjected to different combinations of the same three UV treatments (see Figure 2). The experimental design thus consisted of two different UV treatment periods: an 'acclimatory treatment' of the parent colonies (one lunar month prior to planulation); followed by a 'settlement treatment' of the planulae (two weeks post-planulation). Settlement containers were constructed of round glass dishes, 9 cm in diameter and 2 cm tall, surrounded by reinforced 183 mm Nitex® plankton netting to an overall height of 8 cm. Prior to the experimental period, each container was 'conditioned' in seawater for two weeks, after which loose filamentous algae was removed, leaving behind a fine algal film.

Five containers, each containing 25 randomly-selected planulae, were utilized for each combination of settlement treatment and acclimation treatment, for a total of 45 containers containing 1125 planulae. Containers were submerged in a shallow water table to a depth of 6 cm, such that free exchange of water was allowed through the plankton netting.

The 218 liter shallow water table was supplied with flow-through seawater at a rate of 8.5 liters per minute, with care taken to avoid strong unidirectional currents that might push planulae against one side of the netting.

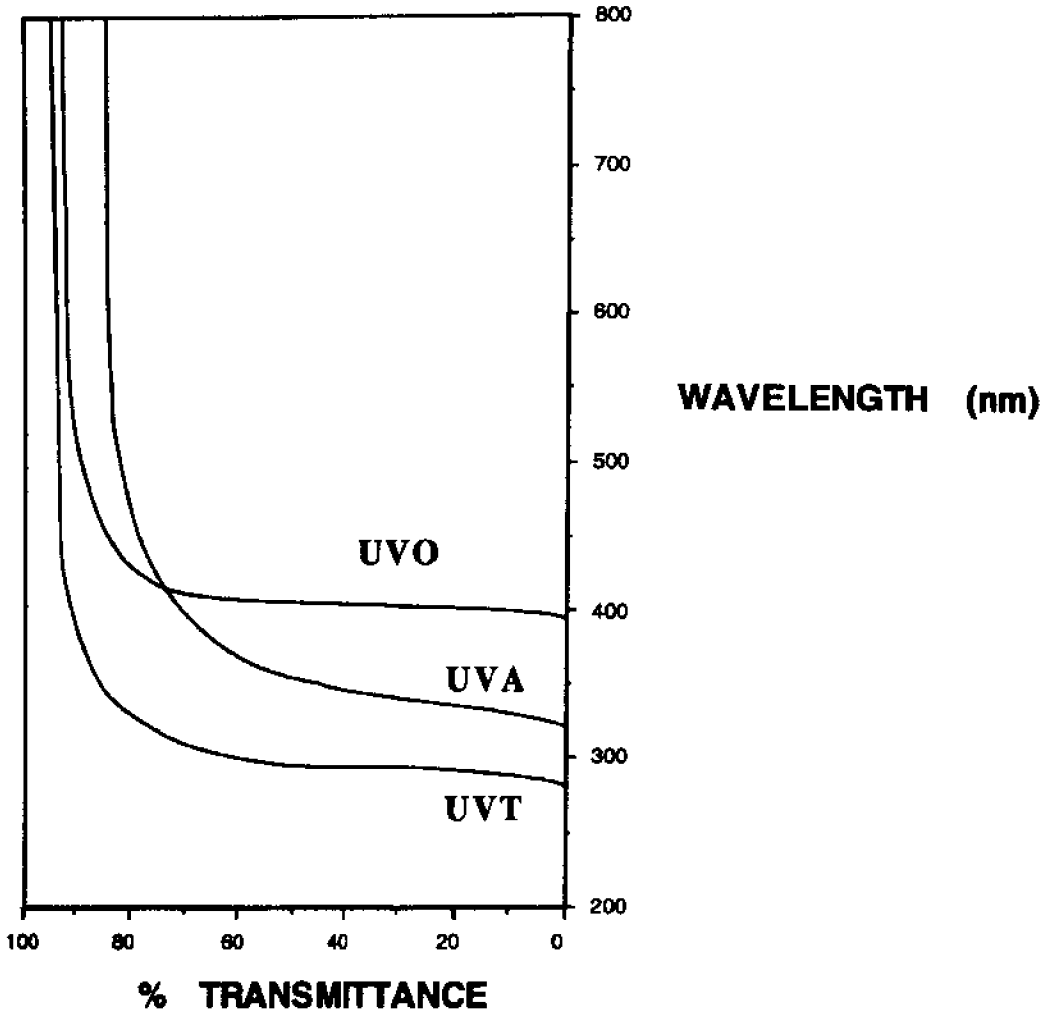

Figure 1. Transmittance characteristics of solar filters as a function of incident wavelength. Filter properties did not change over the period of this study (6 weeks).

The shallow water table was covered at different points with three filters providing UVO, UVA and UVT treatments as before. Tank effects were thus avoided by subdivision of a single tank into three treatments.

The number of planulae settled in each container, as well as the total number of planulae remaining, were counted over a period of 14 days. Settled planulae were those observed to have anchored firmly to the substrate and spread out a basal plate.

RESULTS

Mean larval settlement after 14 days, given as a percentage of the initial 25 planulae, was greatest under the UVO treatment, at 37.3% (±3.7%), compared to 20.8% (±4.9%) and 13.1% (±3.1%) in the UVA and UVT treatments respectively. Maximum settlement after 14 days for any individual container was 80% (observed in the UVA treatment) whilst minimum settlement for any container was 0% (observed in the UVT treatment) (See Fig. 3).

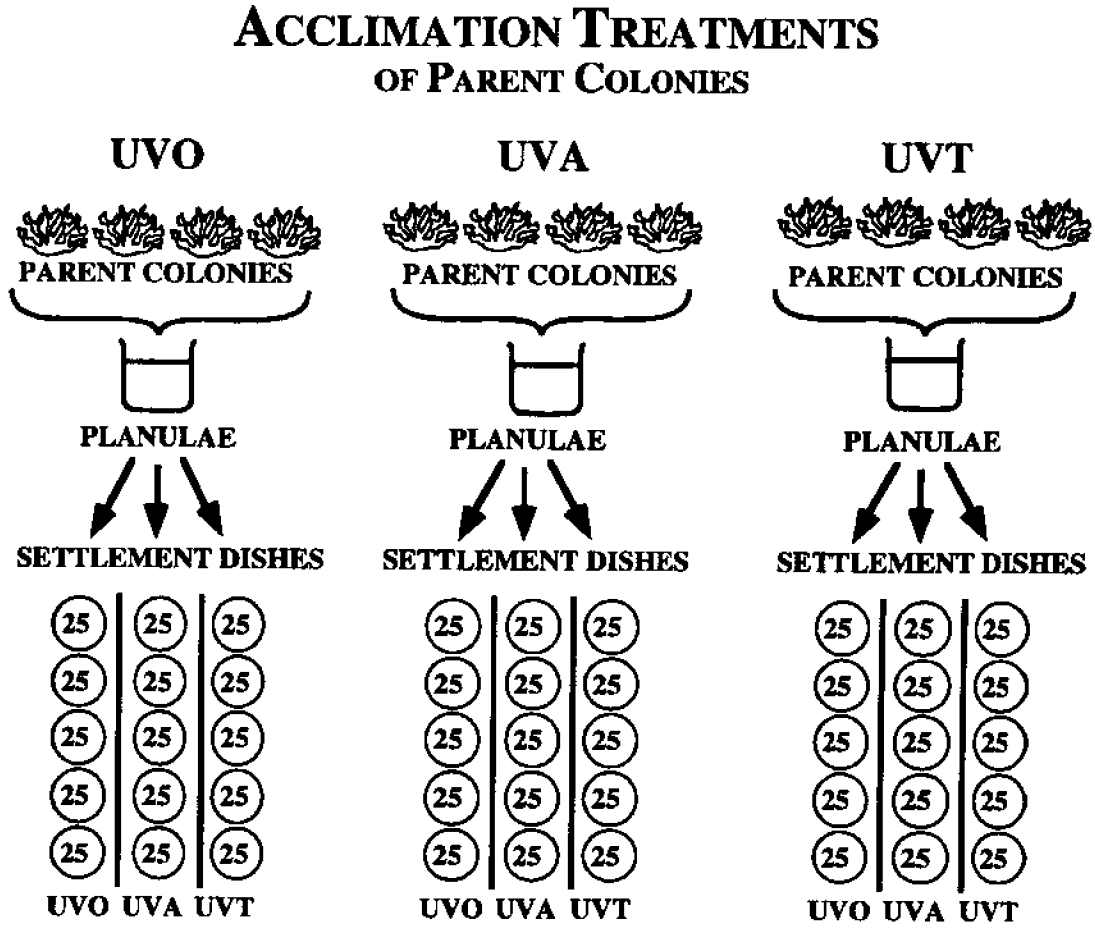

Figure 2. Experimental protocol. Parent colonies are acclimated to different UV treatments for one lunar month prior to planulation. Settling planulae released from the parent colonies are then exposed to different combinations of the same three UV treatments for 14 days.

Mean settlement of larvae originating from parent colonies acclimated to conditions of UVT was 26.7% (±6.0%) after 14 days, whilst for UVA and UVO it was 26.4% (±4.0%) and 18.1% (±3.7%) respectively (See Fig. 4).

Planula mortality, estimated as the loss of planulae from the containers, did not differ between treatments, and did not exceed 25% after 14 days.

When variance in settlement due to UV settlement treatment is removed, acclimatory UV treatment has no effect on planula settlement rate. However, when variance due to acclimatory UV treatment is removed, it can be seen that settlement UV treatment does have a significant effect (Two-way ANOVA at each time interval, see Table II). The effect of UV settlement treatment appears after only two days. There is never any interaction between the two treatments. Settlement rate in the UVO is significantly higher than rates under both UVA and UVT, but there is no significant difference between UVA and UVT (Student Newman-Keuls Test, a = 0.05), this is

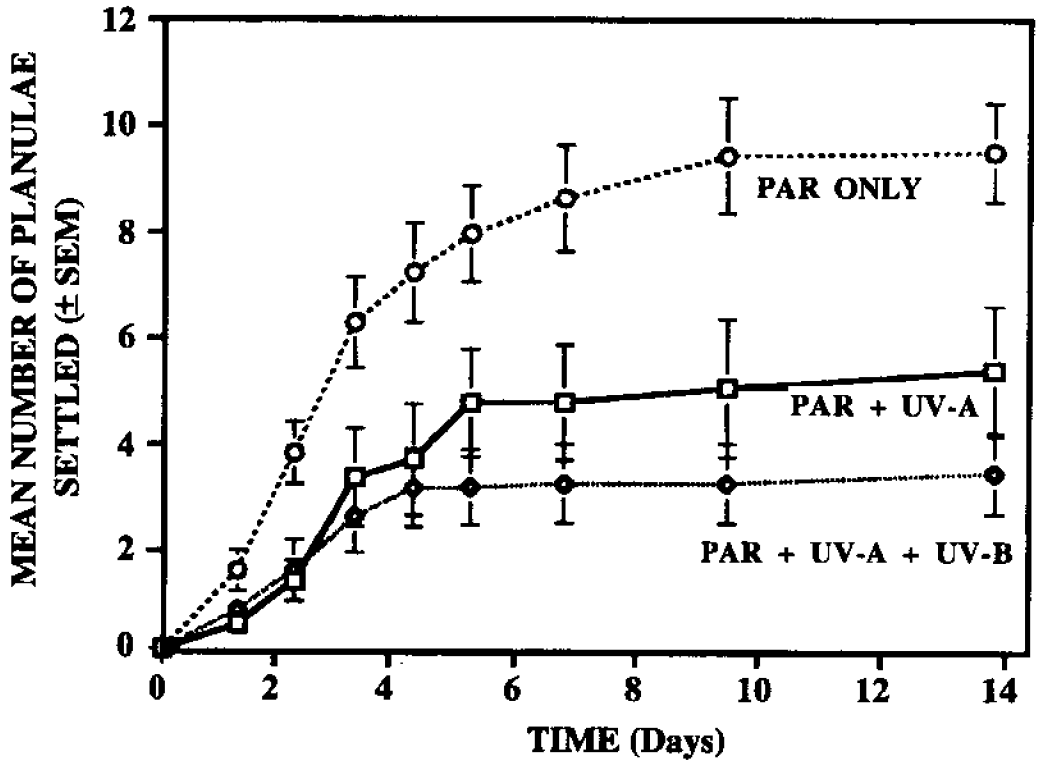

Figure 3. Settlement rate of planula larvae of *Pocillopora damicornis* as a function of UV treatment during settlement (UV acclimatory treatments of adult colonies grouped).

also true from the second day onwards. These results argue for the significance of UV-A radiation as an inhibitory factor in the settlement process, since when UV-B is removed there is no significant increase in settlement (similar curves for UVA and UVT), but when UV-A is removed, settlement increases markedly (significant difference between UVO and both UVA and UVT curves).

An observable decline in pigmentation of the planulae occurred over the 14 day period of study. This decline in pigmentation was observed in all three treatments, and affected both free-swimming and settled planulae. This reduction in coloration made assessments of reverse metamorphosis (Richmond, 1985) problematic; quantification of this phenomenon is not included here.

HPLC Quantification of MAAs

HPLC analysis revealed newly-released planulae to contain at least five different mycosporine-like amino acids (MAAs): mycosporine-glycine (λ_{max} = 310 nm), asterina (λ_{max} = 330 nm), porphyra-334 (λ_{max} = 334 nm), shinorine (λ_{max} = 334 nm) and one unknown, thought to be mycosporine-glycine:valine (λ_{max} = 335 nm). Additionally some samples contained small amounts of palythinol (λ_{max} = 332 nm) and palythene (λ_{max} = 360 nm) (Karentz *et al.*, 1991). Mean concentrations of the five MAAs consistently present, as a function of the UV acclimatory treatment of the parent colony, are given in Table III.

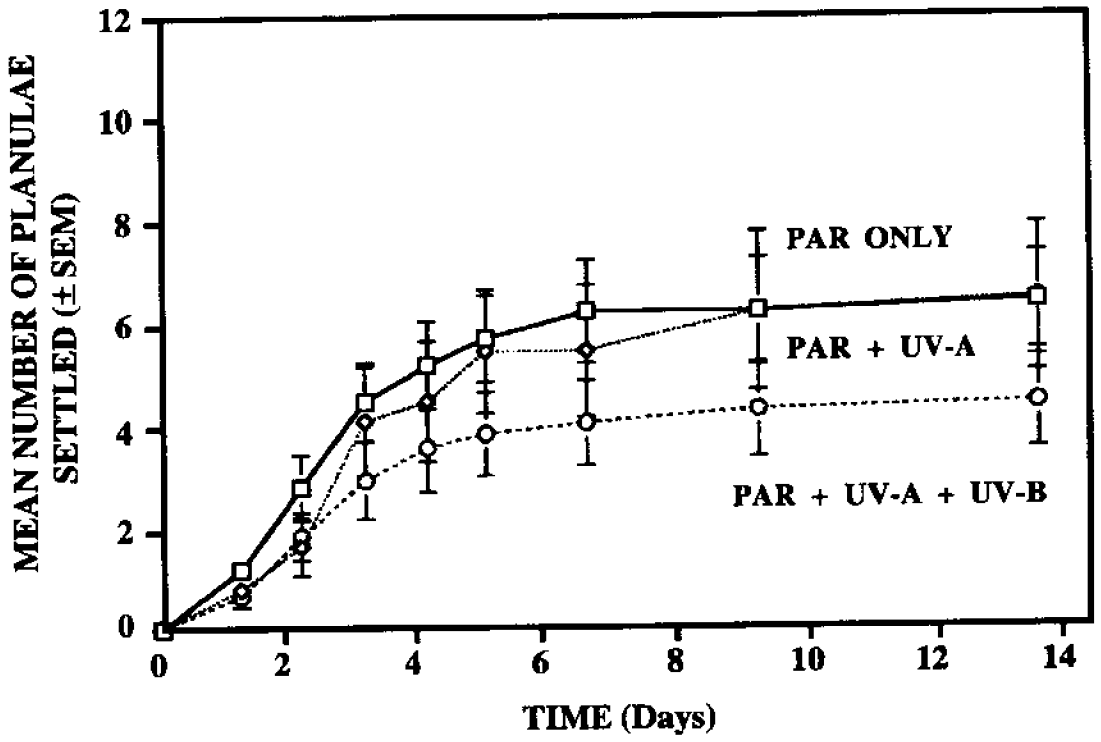

Figure 4. Settlement rate of planula larvae of *Pocillopora damicornis* as a function of UV acclimatory treatment of parent colony (UV settlement treatments of planulae grouped).

Table II. Results of a two-way ANOVA ($\alpha = 0.01$) at each time interval, showing significance of UV Settlement (X) and Acclimation Treatment (Y). In some cases appropriate transformations of the data were made to conform to the conditions of the analysis (as shown).

TIME (Days)	0	1.25	2.208	3.208	4.208	5.167	6.75	9.458	13.875
DATA TRANSFORMATION	-	Square Root	Square Root	NONE	NONE	Square Root	Square Root	Square Root	NONE
SETTLEMENT TREATMENT (X)	-	NO	YES	YES	YES	YES	YES	YES	YES
ACCLIMATORY TREATMENT (Y)	-	NO	NO	NO	NO	NO	NO	NO	NO
INTERACTION (X * Y)	-	NO	NO	NO	NO	NO	NO	NO	NO

Table III. Mean MAA concentrations (in nm/mg protein) as a function of UV acclimatory treatment of parent colony. Mean values are calculated from *n* replicates of 25-50 planulae. Those values marked with an asterisk (*) are not significantly different between treatments (one-way ANOVA, α=0.05). Mean protein content (=14.3 mg per planula) is calculated from Richmond (1982). See also Figure 5, noting logarithmic scale.

		UV ACCLIMATORY TREATMENT		
		UVT *(n=5)*	UVA *(n=7)*	UVO *(n=5)*
MYCOSPORINE-GLYCINE	MEAN CONCENTRATION (nm/mg protein) (±SEM)	1.097 (±0.188)	0.665* (±0.057)	0.511* (±0.032)
SHINORINE	MEAN CONCENTRATION (nm/mg protein) (±SEM)	0.877* (±0.094)	0.724* (±0.114)	0.708* (±0.080)
PORPHYRA-334	MEAN CONCENTRATION (nm/mg protein) (±SEM)	0.484* (±0.041)	0.395* (±0.027)	0.353* (±0.033)
MYCOSPORINE-GLYCINE:VALINE	MEAN CONCENTRATION (nm/mg protein) (±SEM)	15.926* (±0.543)	24.694 (±1.214)	16.282* (±1.168)
ASTERINA	MEAN CONCENTRATION (nm/mg protein) (±SEM)	4.830* (±0.186)	7.594 (±0.429)	4.633* (±0.525)

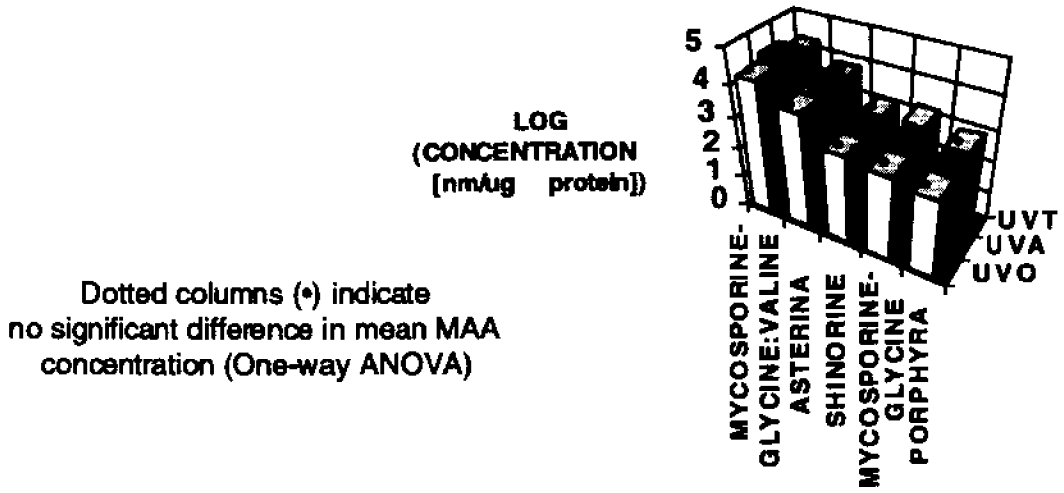

Figure 5. Concentration of MAAs in planulae as a function of UV acclimatory treatment of parent colonies. Note logarithmic scale.

Newly released planulae left for a period of 2-4 days under a variety of UV treatments contained mean concentrations that, in four out of the five MAAs concerned, were significantly less than those analyzed immediately post-release (see Figure 6, noting logarithmic scale). These changes are summarized in Table IV.

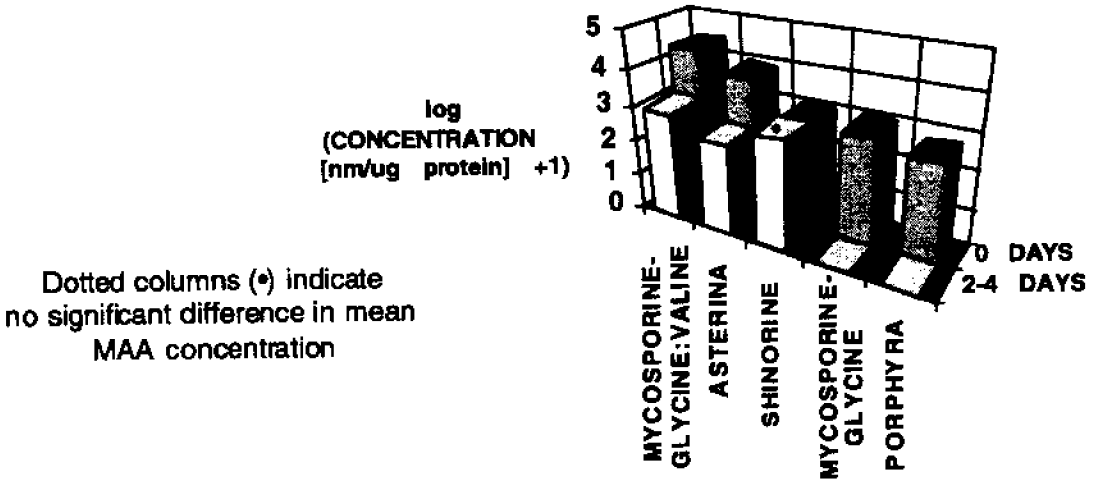

Figure 6. Concentration of MAAs as a function of time after release. Note logarithmic scale.

DISCUSSION

UV-A Inhibition of Settlement

This study demonstrates that settlement of the planula larvae of *Pocillopora damicornis* is inhibited by high-energy solar UV radiation, particularly in the UV-A region of the spectrum. Whilst some influence by UV-B is also suggested, although not at a significant level (see Fig. 3), this effect is heavily outweighed by that of the UV-A. This is in contrast to many studies of larval ecology and reproductive success that have revealed UV-B as the major detrimental or biocidal component in the solar spectrum (Chalker-Scott *et al.*, 1992; Berghahn *et al.*, 1993).

Previous studies of the effect of UV on larval ecology have not always investigated the full range of the UV spectrum, and hypotheses regarding potential action spectra for UV detrimental effects often appear to have been assumed *a priori*. However, it is apparent that the effects of UV-A have been underestimated and underinvestigated. Given that the total radiant flux in the UV-A is typically at least an order of magnitude higher than that of UV-B, it is to be expected that detrimental effects could be greater for this region (see Peterson *et al.*, this volume). Whilst for many processes the energy per quantum is clearly the parameter of interest, emphasizing the higher energy UV-B region of the spectrum, total radiant flux in the lower energy region of the UV spectrum cannot be ignored. This is doubly true when it is observed that the distinction between UV-A and UV-B is arbitrary and bears no direct biological or physical relevance; they represent the division of a continuum into discrete categories.

Additionally, this study reveals 'sublethal' effects of UV radiation that have direct consequences on recruitment (reproductive success) of the parent organism. Differences in settlement under UV treatment not only manifest themselves as differences in the final number of planulae settled, but also as differences in initial rates of settlement. Variation in reproductive success is likely, therefore, to be greater than that estimated solely from the final number of planulae settled, since variation in the time spent in the plankton will also affect overall reproductive success. This is especially true when planktonic predation is intense, or when strong currents sweep planulae off the reef and into the open ocean, where chances of encountering suitable substrate are dramatically reduced.

Table IV. Comparative mean concentrations of MAAs immediately post-release; and after 2-4 days under a variety of UV treatments. Mean values are calculated from *n* replicates of 25-50 planulae. Those values marked with an asterisk (*) are not significantly different (Mann-Whitney U-test, a = 0.05).

		TIME OF ANALYSIS	
		NEWLY-RELEASED (time=0) *(n=17)*	POST-RELEASE (time=2-4 days) *(n=8)*
MYCOSPORINE-GLYCINE	MEAN CONCENTRATION (nm/mg protein) (±SEM)	0.747 (±0.081)	0
SHINORINE	MEAN CONCENTRATION (nm/mg protein) (±SEM)	0.764* (±0.058)	0.932* (±0.150)
PORPHYRA-334	MEAN CONCENTRATION (nm/mg protein) (±SEM)	0.409 (±0.022)	0
MYCOSPORINE-GLYCINE:VALINE	MEAN CONCENTRATION (nm/mg protein) (±SEM)	19.641 (±1.212)	0.906(±0.290)
ASTERINA	MEAN CONCENTRATION (nm/mg protein) (±SEM)	5.910 (±0.419)	0.308 (±0.157)

Effect of UV Acclimatory Treatment

This study fails to reveal any difference in UV tolerance of planulae produced by parent colonies acclimated for one month to different UV regimes (Fig. 4 and Table II). The possibility exists that one reproductive cycle is insufficient to produce a detectable difference in tolerance, and that longer acclimatory periods are required to demonstrate an effect. However, from a theoretical point of view it would appear unlikely that such an effect could exist, since the planula larvae of *P. damicornis* are positively buoyant on release, and would therefore be expected to mix in the upper surface waters, regardless of the depth of their initial release. A more detailed knowledge of planktonic larval dynamics and temporal changes in larval buoyancy associated with lipid decline, are required to adequately resolve these issues (but see Richmond, 1987).

Ecological relevance of these results

This study investigates solar radiant flux, without artificial increase of the solar spectrum. In contrast to much of the literature regarding the biological effects of UV, maximum dose rates used in this study typically underestimate maximum rates likely to be encountered in the field, since the filters maximum transmittance never exceeds 92% (see Figure 1). However, mean dose over a 14-day period in any region of the spectrum may be more than the mean dose received in the field, since in natural scenarios planulae may be transported to greater depths with lower UV dosage. Nevertheless, both dose and dose rate lie within the region of values that possess potential ecological relevance.

A decrease in the earth's protective ozone layer, and a concomitant increase in solar UV penetration will increase both the amount and the spectral quality of UV reaching the earth's surface (Calkins, 1982; Worrest and Caldwell, 1986). Significant increases in UV-A under this

scenario are unlikely. Hence if reproductive success of planulating corals is heavily affected by incident UV-A, but only marginally affected by UV-B, further reductions in recruitment due to ozone depletion are unlikely.

P. damicornis is found only on reef flats at shallow depths of 0 - 3 m in Kane'ohe Bay, where the specimens used in this study were collected. Dominant corals co-occurring at these depths are *Porites compressa*, and branching *Montipora* spp. From its observed depth distribution it might be hypothesized that developing colonies of *P. damicornis* compete well under high radiation conditions, but avoid the initial inhibitory effects of UV-A by settling in the many cryptic microhabitats present on the reef flat. Distribution at greater depths may be limited by the marked reduction in PAR in the relatively turbid waters of Kane'ohe Bay.

The possibility exists that UV radiation is not having an effect on the planulae *per se*, but rather on the substrate for which they select. For example, the absence of UV might affect a particular algal turf, causing it to produce a certain chemical stimulus that encourages settlement (Morse & Morse, 1991). Such a hypothesis, if true, would indicate only an indirect effect of UV on planulae. However, it nevertheless supports the observation that in ecological scenarios UV radiation is an important factor determining settlement site selection. In cases where either cryptic sites are few, or settling time limited due to strong currents off the reef, settlement site selection would still play a major role in recruitment success.

Ecological Significance of MAAs

This study reveals significant differences in the concentrations of some MAAs in planula larvae as a function of the UV treatment to which the parent coral colonies were exposed. Parent colonies exposed to UV radiation produced planulae containing more MAAs than colonies screened from UV. However, the finer scale spectral differences produced more counterintuitive results.

When parent colonies were exposed to UVA conditions, i.e. a combination of PAR and UV-A, they produced planulae with higher concentrations of both mycosporine-glycine:valine and asterina than colonies exposed to UVT or UVO treatments. However, when parent colonies were exposed to UVT, i.e. PAR, UV-A and UV-B, they produced more mycosporine-glycine than colonies exposed to UVA or UVO treatments. This differential variation in mean MAA concentration can be interpreted as a function of both the intensity and spectral composition of the UV treatment, as well as the particular absorbance characteristics of the MAA concerned (see Fig. 7).

Mycosporine-glycine has a peak absorbance (λ_{max}) at 310 nm, in the UV-B; whilst mycosporine-glycine:valine and asterina have absorbance peaks at 335 nm and 330 nm respectively, which are both in the UV-A (see Fig. 7). Hence it would appear that colonies exposed to conditions of full UV (UV-A and UV-B) preferentially invest in MAAs blocking in the (more damaging) UV-B, whilst colonies exposed only to UV-A place all their investment in UV-A blockers.

However, this is not predicted by the settlement observations presented earlier, which showed UV-A to be the region of the UV spectrum causing most of the settlement inhibition. If UV-A were truly the ecologically most damaging component of solar radiation, then parent colonies exposed to UV-A and UV-B, rather than UV-A alone, would not be expected to withdraw investment in UV-A blockers for placement in UV-B blockers.

Additionally, despite significant variation (1.5 to 2-fold) in mean concentration of some MAAs as a function of UV treatment level, no significant differences in settlement were found as a function of the same treatment levels (Figure 4 and Table II). These results do not indicate a protective function of MAAs, at least at the ecological resolution of this study. Care must therefore be taken to distinguish variation in MAA content in response to UV radiation, from the assumption that MAAs must have a protective function and, therefore, have adaptive value.

The sharp reduction in MAA concentration in planulae analyzed 2-4 days after release raises important questions. If MAAs are indeed providing a protective function, it is unlikely that such a marked reduction in UV blockers could occur in ecological scenarios, since planulae are known to be competent over time scales far exceeding these (Richmond, 1987). One alternate possibility is that the reduction in MAAs detected during this study is due to the observed bleaching of planulae mentioned earlier, since if MAAs are synthesized by the zooxanthellae and not the coral itself, then a reduction in zooxanthella abundance (or competence) associated with bleaching might cause a concomitant decrease in MAAs. On the other hand, if MAAs are not directly

providing a protective function, and merely arise as a result of processes only secondarily associated with UV, then dramatic decreases in MAA concentration over ecologically short time scales provide no obstacles to our conceptual framework.

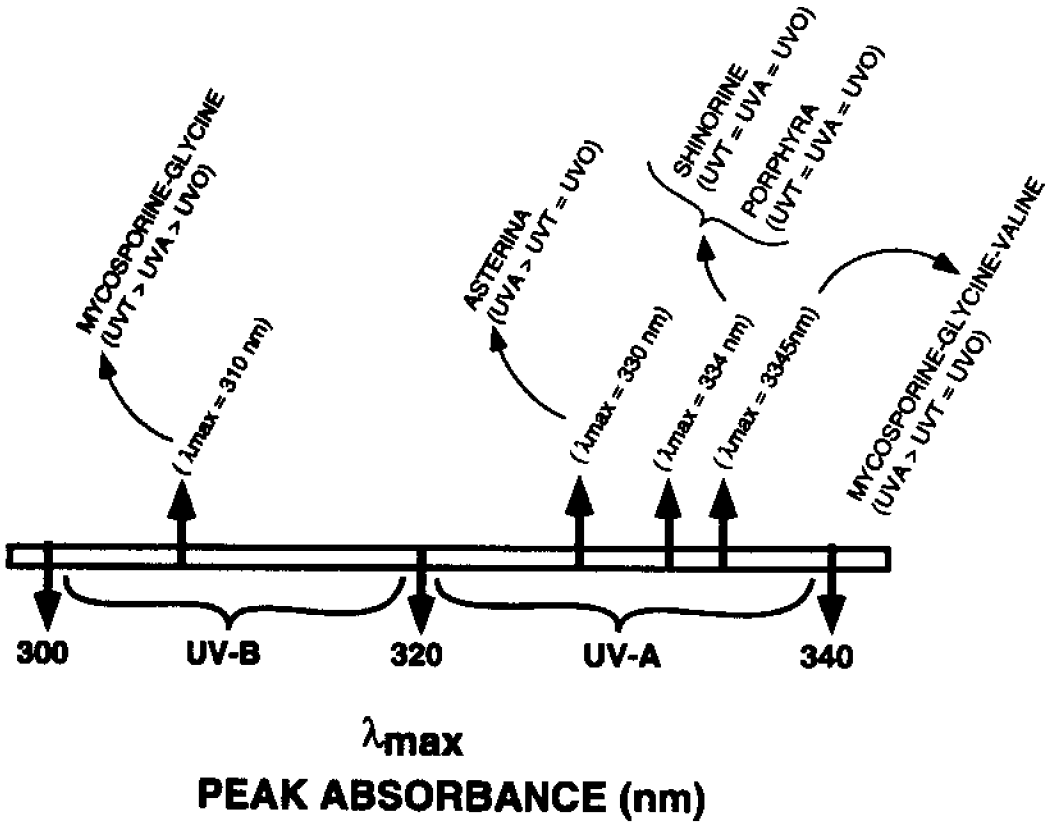

Figure 7. Peak absorbance (λ_{max}) of the five MAAs found within the planula larvae of *Pocillopora damicornis*. Relationships in parentheses refer to significant differences in mean concentration as a function of UV acclimatory treatment of the parent colony.

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Ultraviolet radiation: helpful or harmful to growth of cultured zooxanthellae?

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ABSTRACT: Typical *in vitro* culturing techniques of microalgae employ the use of fluorescent lighting, which produces ultraviolet (UV) radiation. Zooxanthellae cultures from the sea anemone *Aiptasia pulchella* were used to test the hypothesis that UV produced by fluorescent bulbs used in laboratory culturing may bedetrimental to their growth. Aliquots of forty milliliters of diluted culture were placed under a lighting system that consisted of four 30 watt Westinghouse brand "Cool White" fluorescent bulbs on a 12:12 hour light:dark photoperiod. This system provided an total irradiance of 18.5 W $m^{-2}s^{-1}$ (0.0467 W $m^{-2}s^{-1}$ at 300 - 320 nm (UV-B), 0.498 W $m^{-2}s^{-1}$ at 320 - 400 nm (UV-A) and 18.0 W $m^{-2}s^{-1}$ at 400 - 700 nm (PAR), respectively). The hypothesis was tested by subjecting 3 Pyrex® test tubes of zooxanthellae culture to each of the following regimes: full spectrum radiation provided by the fluorescent bulbs, fluorescent radiation lacking UV-B (300 - 320 nm), and fluorescent radiation lacking UV-B and UV-A (320 - 400 nm). Pyrex® was chosen for two reasons: 1) because of its low reduction (13%) of biologically effective radiation (Smith & Baker, 1980), and 2) its common use in microalgae culture. The first tray was fitted with chloroflurocarbon (Aclar®), which transmits wavelengths greater than 280 nm. The second tray was fitted with a cover of polyester film (DuPont Mylar®) that blocked out wavelengths below 320 nm but transmitted most of the longer wavelengthradiation. The third tray was fitted with a cover of UV0 (polycarbonate), which was opaque to both UV-B and UV-A radiation. Culture tubes were laid at a 45° angle; this configuration allowed cultures to receive unshaded irradiance throughout the 12 hour light period. Cell counts were conducted using a hemocytometer and light microscopy every 3 days over the 15 day experimental period. Eight chamber counts per test tubewere taken to provide estimated population densities of zooxanthellae. Data from two runs were tested using ANOVA; no significant difference was found between the three treatments. These results suggest that zooxanthellae raised *in vitro* are not affected by fluorescent-produced UV.

INTRODUCTION

Interest in the ecological importance of solar ultraviolet (UV) radiation has been stimulated by concern over the possible disruption of the earth's protective ozone layer by anthropogenic atmospheric pollution. UV radiation measurements taken by Jerlov (1950) showed that clear, oceanic waters, such as those found over many coral reefs, transmit considerable amounts of UV radiation, yet the previously held view that UV is without ecological importance in all types of waters remained predominant for a considerable period. However, in the last 15 years, numerous observations have given extensive support to Jerlov's view of UV's importance in aquatic environments (see Calkins, 1982 for review). In particular, UV radiation at and above ambient levels has been found to be harmful to many forms of microalgae (McMinn *et al.*, 1994; Cullen et al., 1992; Cullen & Lesser, 1991). Effects of serious UV radiation damage range from reductions in carbon assimilation (Hazzard, 1990) to "bleaching" of chlorophyll and reduction of cell motility (Gerber & Haeder, 1993).

Hermatypic, or reef-building, corals are predominantly found in the euphotic zone of warm, tropical oceans and seas (Falkowski *et al.*, 1990). Their success is partly due to their symbiotic relationship with zooxanthellae (*Symbiodinium* sp.), single-celled dinoflagellate algae that live within the endodermal tissue of the coral animal. These algae require light for photosynthesis, and provide the animal partner with photosynthetic products that contribute to nutrition (Muscatine & Porter, 1977) and aid in the construction of the coral's calcium carbonate skeleton (Goreau, 1961; Pearse & Muscatine, 1971). Zooxanthellae have been isolated from symbiotic partnerships and cultured *in vitro* successfully (Jokiel & York, 1982; Lesser & Shick, 1989). Jokiel and York (1984) demonstrated that *in vitro* cultures of zooxanthellae grown under natural irradiance unshielded from UV were severely impaired in ways similar to other microalgae.

Typical *in vitro* culturing techniques employ the use of fluorescent lighting, which produces both UV-A and UV-B. The experiment described here was designed to test the hypothesis that fluorescent lighting may impair the growth of laboratory cultures of zooxanthellae. If the UV produced by fluorescent lighting is eliminated by using one, or a combination, of filters, it is hypothesized that zooxanthellae division rates will increase.

MATERIALS AND METHODS

Culture of zooxanthellae

Zooxanthellae (*Symbiodinium* sp.) from the sea anemone *Aiptasia pulchella* was obtained from the Coconut Island zooxanthellae collection. All cultures were maintained in Pyrex® test tubes (25 x 150 mm diameter). Pyrex® was chosen for two reasons: 1) because of its low reduction (13%) of biologically effective radiation (Smith & Baker, 1980), and 2) its common use in microalgae culture. The culture tubes were washed with 10% Liqui-Nox®, rinsed with tap water, rinsed with 10% HCl, rinsed with distilled water, stoppered with cotton and autoclaved. One liter of 0.45 micron filtered seawater was filter-sterilized using 0.22 micron Millipore® filter paper and enriched to make "f/2" medium. An initial cell count was made on the Coconut Island master culture using a hemocytometer. A dilution was made from this master culture, and aliquots of forty milliliters, containing a known zooxanthellae density, were placed in the sterile Pyrex® and stoppered with sterile cotton. The cotton stoppers were capped with Parafilm® before the tubes were placed under their respective irradiance regimes.

Lighting

The lighting system used consisted of four 30 watt Westinghouse "Cool White" fluorescent bulbs on a 12:12 hour light:dark photoperiod, which is typically used for *in vitro* microalgae culture. This system provided an total irradiance of 18.5 W $m^{-2}s^{-1}$ (0.047 W $m^{-2}s^{-1}$ at 300 - 320 nm (UV-B), 0.498 W $m^{-2}s^{-1}$ at 320 - 400 nm (UV-A) and 18.0 W $m^{-2}s^{-1}$ at 400 - 700 nm (PAR)), respectively, when measured using a Li-Cor LI-1800UW Underwater Spectroradiometer at Coconut Island, Kane'ohe, Hawai'i on June 16, 1994. Nine test tubes of zooxanthellae culture were split among three treatments. These treatments were: full spectrum radiation provided by the fluorescent bulbs, fluorescent radiation lacking UV-B (300-320 nm), and fluorescent radiation lacking UV-B and UV-A (320-400 nm). The first tray was fitted with chloroflurocarbon (Aclar®), which transmits wavelengths greater than 280 nm. The second tray was fitted with a cover of polyester film (DuPont Mylar®) that blocks out wavelengths below 320 nm but transmits most of the longer wavelength radiation. The third tray was fitted with a cover of UVO (polycarbonate), which is opaque to both UV-B and UV-A radiation. Culture tubes were laid at a 45° angle; this configuration allowed cultures to receive unshaded irradiance throughout the 12 hour photoperiod.

Zooxanthellae cell counts

Cell counts were conducted using a hemocytometer and light microscopy approximately every three days over the 15 day experimental period. Eight chamber counts per test tube were taken to provide estimated population densities. Test tubes were agitated each time a sample drop was extracted to ensure homogeneous mixing of each culture. Cell count data was subjected to ANOVA to determine if there was a difference between cultures of each treatment for each sample day.

RESULTS AND DISCUSSION

Two runs were conducted to test the hypothesis that fluorescent lighting UV may impair *in vitro* cultures of zooxanthellae. Cultures tended to concentrate in areas nearest the irradiance source when left undisturbed. Cells in both motile and non-motile phases were observed during cell counts. Initial cell density per ml for each run was 7000 and 8000, respectively. Over the course of the experiment, cell density increased exponentially for the first twelve days of the experiment before leveling off between Day 12 and Day 15 (Figs. 1 and 2). This leveling off of density may have been a result of cultures reaching senescence. ANOVA was used to determine if the differences between treatments were significant. Differences were found to be not significant.

Cultures grown under the full UV treatment showed slightly higher densities for a majority of the experiment. Jokiel and York (1984) also observed this phenomenon when *Symbiodinium microadriaticum*, *Phaeodactylum tricornutum* and *Tetraselmis* sp. cultures where grown under conditions of light limitation (6% intensity of natural sunlight). One explanation for this higher density is that some species of algae use UV radiation photosynthetically under light-limited growth conditions (Jokiel & York, 1984). Haildal (1968) demonstrated that the action spectrum for photosynthesis in *S. microadriaticum* shows oxygen production in the UV-A range.

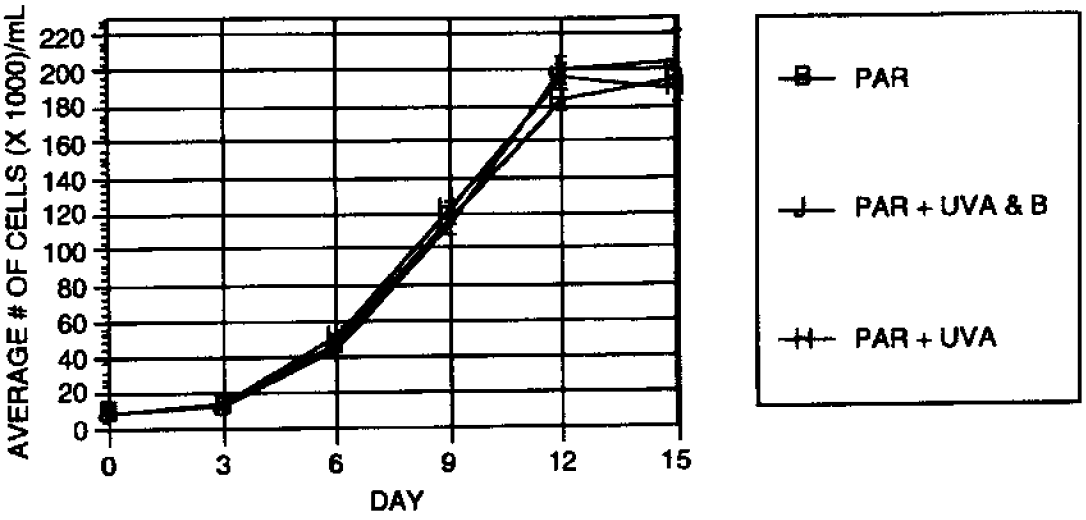

Figure 1. First Run. Zooxanthellae densities when grown under different treatments of PAR, PAR + UV-A, and PAR + UV-A + UV-B.

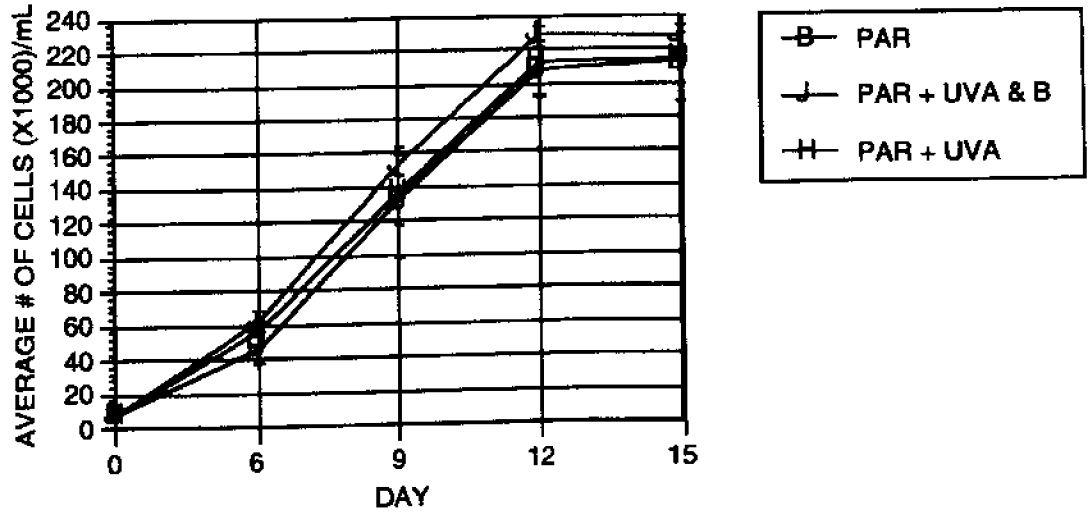

Figure 2:. Second Run. Zooxanthellae densities when grown under different treatments of PAR, PAR + UV-A, and PAR + UV-A + UV-B.

These results, although preliminary, suggest that our stock cultures are not being adversely affected by the UV radiation produced by flourescent lamps. However, the cultures used in this experiment were isolated many years ago and given the rapid division rate of these organisms, untold generations have occurred under these conditions. Therefore, selection for resistant forms might have occurred in these cultures. This could result in the selection of cultures that are UV-tolerant and non-representative of natural populations. The next logical experiment would be to compare response of freshly isolated zooxanthellae of this type with the laboratory forms that have been grown in culture under fluorescent lights since they were isolated from their host over 15 years ago. On the other hand, Jokiel and York (1982, 1984) used the same strain in their work and found the cultured cells to be sensitive to solar UV-A and UV-B. More work dealing with subtleties other than growth needs to be conducted, including analyzes of pigment composition and concentration, to ensure that cultured zooxanthellae are an adequate representation of natural populations.

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Survey of mycosporine-like amino acids in macrophytes of Kane'ohe Bay

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ABSTRACT: Mycosporine-like amino acids (MAAs) were surveyed by reverse-phase high performance liquid chromatography (HPLC) in 19 species of Rhodophyta (red algae), 11 species of Phaeophyta (brown algae), 8 species of Chlorophyta (green algae), and 3 species of Cyanophyta (blue-green algae). Seven MAAs were identified and all but three species of chlorophytes and two species of phaeophytes contained at least one MAA. All of the MAAs detected have been observed previously in marine organisms. Mycosporine-glycine (λ_{max} = 310 nm) and shinorine (λ_{max} = 334 nm) were the most abundant MAAs, both being found in 54.9% (28/51) of the species studied. Porphyra-334 (λ_{max} = 334 nm) was found in 31.4% (16/51) of the species studied, palythine (λ_{max} = 320 nm) was found in 19.6% (10/51) and asterina-330 (λ_{max} = 330 nm) was found in 15.7% (8/51) species studied. The least abundant MAAs detected were palythinol, which was found in 3/51 species, and palythene which was found in only one species studied.

INTRODUCTION

Over the last several decades there has been an increased interest in the potential for enhanced biologically effective ultraviolet (UV) radiation impinging on the earth's surface. Solar radiation reaching the earth's surface is composed of both photosynthetically active radiation (PAR, 400 - 700 nm) and UV radiation (290 - 400 nm). Photosynthetic organisms which require PAR for primary production are also exposed to the potentially damaging effects of UV radiation. The high transparency of tropical ocean water to solar PAR and UV radiation suggests that shallow-water dwelling organisms are exposed to high fluxes of UV radiation, possibly requiring a trade-off between the requirements for maintaining high rates of photosynthesis while preventing damage from UV radiation.

Biochemical defenses against the damaging effects of UV radiation include the presence of UV-absorbing compounds known as mycosporine-like amino acids (MAAs). These compounds, first identified as 'S-320' compounds in corals and a cyanobacterium (Shibata, 1969), have since been found in a wide variety of marine organisms spanning cyanobacteria (Shibata, 1969) to teleosts (Dunlap *et al.*, 1989) and ranging geographically from tropical (Dunlap *et al.*, 1986) to Antarctic waters (Karentz *et al.*, 1991). MAAs have absorbance maxima ranging from 310 - 360 nm and together may provide a broad-band filter to UV radiation. The photoprotective function of these compounds has been inferred from their UV absorption porperties (Price & Forrest, 1969; Shibata, 1969; Dunlap *et al.*, 1986), the concentration of these compounds in coral reef-dwelling species (Shibata, 1969; Dunlap *et al.*, 1986), the positive correlation between the 325 nm peak in *Porites lobata* with light intensity (Maragos, 1972), the decrease in concentration of these compounds as depth increases in coral species (Dunlap *et al.*, 1986; Scelfo, 1986; Shick *et al.*, 1991), the modulation of the concentration of these compounds by exposure to UV radiation (Shick *et al.*, 1991), and that corals grown in full spectrum solar radiation produce higher concentrations of 'S-320' (MAAs) than those screened from UV radiation (Jokiel, 1980; Jokiel & York, 1982). The production of these compounds has been shown to be induced in the presence of UV radiation in the dinoflagellate *Symbiodinium microadriaticum* , symbiotic with the jellyfish *Cassiopeia xamachana* (Banaszak, 1994). Although the pathway for these compounds is restricted to bacteria, fungi and algae, these compounds may be passed through the food chain to higher trophic levels as was found in holothurians feeding on bacteria (Shick *et al.*, 1992).

Macrophytes are important biological contributors of primary productivity and biomass in ecosystems such as coral reefs and fleshy species of algae serve as the base of food chains which may also serve as a source of UV-absorbing compounds for invertebrates and vertebrates. Calcareous algae are also important geological contributers to coral reefs by sediment formation

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from the breakdown of the skeleton which aids in the build up of the structure of reefs.

The purpose of this study was to survey the MAAs in macrophytes from Kane'ohe Bay, Hawai'i and to determine the taxonomic distribution of these compounds.

MATERIALS AND METHODS

Macrophytes were collected in June and July, 1994 during daylight hours by SCUBA diving or snorkelling at depths of one to two metres at patch reefs in Kane'ohe Bay, Hawai'i (21° 29' N, 157° 50' W) representing turbid, tropical waters. All specimens were collected from fully exposed and unshaded habitats. Underwater measurements of UV and PAR radiation were taken at the point of collection close to noon on sunny, cloudless days. The measurements were made using a Li-Cor 1800 UW spectroradiometer equipped with a 180° cosine corrected sensor. Measurements were taken at 2 nm intervals at a range of 300 to 850 nm. Three scans were taken at each site and averaged.

The specimens were rinsed in sea water, cleaned to remove epiphytes and other organisms and frozen at -50°C until required for further analysis. For analysis by high performance liquid chromatography (HPLC), approximately one gram of wet tissue was extracted overnight in 5 ml of 100% HPLC grade methanol. MAAs were separated by reverse-phase, isocratic HPLC on a Brownlee RP-8 column (Spheri-5, 4.6 mm ID x 250 mm) which was protected with an RP-8 guard column (Spheri-5, 4.6 mm ID x 30 mm). The mobile phase consisted of 40% methanol (v:v), 0.1% glacial acetic acid (v:v) in water and run at a flow rate of 0.6 ml min^{-1}. Detection of the peaks was carried out by UV absorbance at 313 nm and 340 nm using a diode array absorbance detector. Standards for seven MAAs were provided courtesy of Dr. Deneb Karentz. Identities of peaks were confirmed by the ratios of 313 nm to 340 nm absorbances and by the maximal wavelength of absorbance. The concentrations of the known compounds are expressed in nanomoles per mg protein. There were two unknowns found and these are expressed as a percentage of the total peak area. Protein measurements were determined using the Lowry technique with the Hartree modification (Hartree, 1972) on the methanol extracts used for the determination of the concentration of MAAs.

RESULTS

Figure 1 shows the spectral irradiance profile for Kane'ohe Bay on a sunny cloudless day for various depths. The amount of UV-B, integrated from 300 - 320 nm, ranged from 1.2 to 2.9 W m^{-2} at the depth of collection and UV-A, integrated from 320 - 400 nm, ranged from 27.4 to 53.0 W m^{-2}.

Table I shows the distribution of MAAs in the four divisions of algae studied. There appear to be some trends in the taxonomic distribution of MAAs in the various divisions with red algae having wider varieties of MAAs than green algae, brown algae or cyanobacteria. The most common MAAs found are mycosporine-glycine which absorbs maximally at 310 nm, shinorine which absorbs maximally at 334 nm and porphyra-334 which also absorbs maximally at 334 nm. Palythene, which absorbs at 360 nm, is rarely present possibly because long UV-A wavelengths are required to induce repair mechanisms. This table gives a broad overview of the distribution of these compounds but does not contain the concentrations of these compounds.

The Chlorophyta (green algae) in general contain fewer MAAs than the other divisions and lower concentrations of the various MAAs found in the different divisions (Table II). There is also an unknown which consistently appears. *Halimeda*, the calcareous algal genus, contains an unknown almost exclusively.

The Rhodophyta (red algae) contain the highest concentrations of MAAs and in general exhibit a wider variety of compounds with few exceptions (Table III). The unknown 1 also appears in this division as does another unknown although both make a low contribution relative to the total peak area. The calcareous red algae contain high concentrations of MAAs although they are not as diverse as in the fleshy rhodophytes.

The Phaeophyta (brown algae) contain high concentrations of MAAs but do not exhibit the diversity of MAAs found in the red algae (Table IV). Again, the unknown-1 and -2 show up with the former occurring in a large number of phaeophytes surveyed.

The Cyanophyta (blue-green bacteria) contain relatively low concentrations of MAAs with the unknowns 1 and 2 appearing again (Table V). Unknown 2 appears in higher percentage in cyanobacteria than it did in any other division.

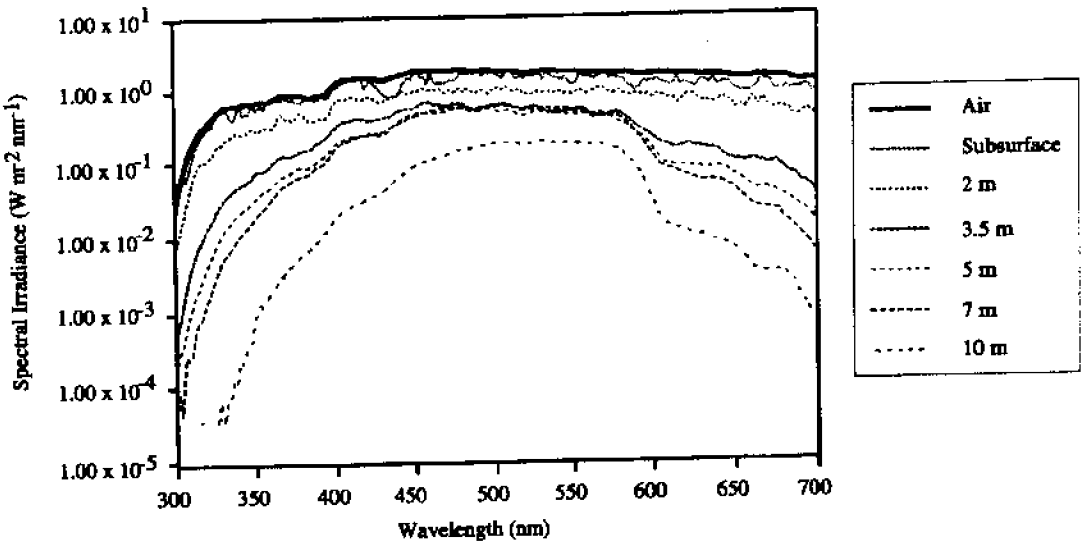

Figure 1. Spectral irradiance depth profile for Kane'ohe Bay on a cloudless day.

DISCUSSION

At least one MAA was found in 90.2% (46/51) species examined. Seven specific MAAs were detected with the most abundant MAAs being mycosporine-glycine and shinorine. Mycosporine-glycine (λ_{max} = 310 nm) and shinorine (λ_{max} = 334 nm) were found in 55% of the species studied. Porphyra-334 (λ_{max} = 334 nm) was found in 31% of the species studied, palythine (λ_{max} = 320 nm) was found in 19% and asterina-330 (λ_{max} = 330 nm) was found in 15% species studied. The least common MAAs detected were palythinol, which was found in 6% species, and palythene, which was found in only one species studied. None of the species examined contained all seven MAAs, however a number of species contained five or six of the MAAs detected. In Antarctic macrophytes, the most common MAAs found were palythine and shinorine (Karentz *et al.*, 1991).

Rhodophyte and phaeophyte species are found more commonly on the patch reefs in Kane'ohe Bay than chlorophyte and cyanophyte species. Generally, chlorophytes when found are present in low biomass, whereas the red and brown algae are more abundant which may be related to the presence of UV-absorbing compounds in the tissues as protection against the damaging effects of UV radiation. There are two unknowns which consistently show up in samples of algae spanning all of the divisions. These compounds need to be identified and the chemical structures elucidated.

The observation that red and brown algae which are more abundant on reefs have more diverse MAAs and higher concentrations of these UV-absorbing compounds is purely

correlative. What is really needed is to demonstrate cause and effect in the effectiveness of these compounds in photoprotection. We know that MAAs absorb UV, but what happens to the energy? To be photoprotective, these compounds need to convert the light energy to some other form of energy (heat or fluorescence) or to dissipate the energy by a cis-trans movement such as is found in carotenoids.

The pathway(s) of MAAs also need to be determined. The problem involved in studying this pathway is that it is so complex and so intricately involved in so many other metabolic pathways that this will be very difficult. A good starting point, though, is to look in species in which these compounds are induced in the presence of UV.

Long-term acclimation studies will be very important in determining the ability of species to acclimate to changing UV conditions such as the effect of increased UV radiation on communities. Green algae, which do not seem to contain UV-absorbing compounds, may be a good indicator species of effects of enhanced UV radiation. Comparison with red or brown algal species may then be a useful parameter to study community effects. In this context we need experiments which will complement the measurement of UV radiation in tropical coastal ecosystems.

Table I. Distribution of mycosporine-like amino acids in macrophytes of Kaneohe Bay.

Sample	Mycosporine-glycine	Shinorine	Porphyra-334	Palythine	Asterina-330	Palythinol	Palythene
CHLOROPHYTA							
Bryopsis sp.	x	x	x				
Caulerpa sertularioides	x				x		
Dictyosphaeria cavernosa							
Dictyosphaeria versluysii	x	x	x				
Enteromorpha							
Halimeda discoidea							
Microdictyon japonicum		x	x				
Neomeris annulata	x	x					
RHODOPHYTA							
Acanthophora spicifera	x	x	x	x	x	x	
Actinotrichia fragilis	x	x					
Centroceras clavulatum	x	x					
Galaxaura rugosa		x	x				
Gelidiella acerosa	x	x	x	x			x
Gracilaria parvispora	x	x	x	x			
Gracilaria salicornia	x	x	x	x	x		
Hydrolithon reinboldii	x	x	x	x	x		
Hypnea cervicornis	x	x		x		x	
Kallymenia sessilis	x						
Laurencia majuscula	x	x					
Laurencia succisa	x	x	x	x	x		
Liagora tetrasporifera		x					
Plocamium sandvicense		x					
Porolithon gardineri	x	x	x	x	x		
Predaea weldii	x	x					
Spyridia filamentosa	x						
Tolypiocladia glomerulata	x		x				
Trichogloea requienii		x					
PHAEOPHYTA							
Colpomenia sinuosa	x	x					
Dictyopteris australis	x	x	x				
Dictyota acutiloba	x	x					
Dictyota bartayresii	x						
Dictyota sandvicensis	x						
Hydroclathrus clathratus	x						
Kappaphycus alvarezii	x	x	x	x	x		
Padina japonica	x	x	x				
Sargassum echinocarpus		x					
Sargassum polyphyllum							
Turbinaria ornata							

Table I. Distribution of mycosporine-like amino acids in macrophytes of Kaneohe Bay (cont.).

Sample	Mycosporine-glycine	Shinorine	Porphyra-334	Palythine	Asterina-330	Palythinol	Palythene
CYANOPHYTA							
Hormothamnion enteromorphoides	x	x	x	x			
Lyngbye majuscula		x			x		
Lyngbye sp.						x	

Table II. Concentrations of mycosporine-like amino acids in fleshy and calcareous green algae.

Sample	Mycosporine-glycine	Shinorine	Porphyra-334	Palythine	Asterina-330	Palythinol	Palythene	Unknown 1	Unknown 2
	(nanamoles mg^{-1} protein)							(% Total peak)	
CHLOROPHYTA-fleshy									
Bryopsis sp.	0.33	0.49	1.25	-	-	-	-	37	-
Caulerpa sertularioides	0.62	-	-	-	0.36	-	-	39	-
Dictyosphaeria cavernosa	-	-	-	-	-	-	-	2	-
Dictyosphaeria versluysii	59.00	37.73	22.11	-	-	-	-	12	-
Enteromorpha	-	-	-	-	-	-	-	100	-
Microdictyon japonicum	-	1.13	0.33	-	-	-	-	27	-
Neomeris annulata	37.45	6.35	-	-	-	-	-	9	-
CHLOROPHYTA-calcareous									
Halimeda discoidea	-	-	-	-	-	-	-	1	-

- = Not Detected

Table III. Concentrations of mycosporine-like amino acids in fleshy and calcareous red algae.

Sample	Mycosporine-glycine	Shinorine	Porphyra-334	Palythine	Asterina-330	Palythinol	Palythene	Unknown 1 (% Total peak)	Unknown 2 (% Total peak)
	(nanamoles mg^{-1} protein)								
RHODOPHYTA-fleshy	-	-	-	-	-	-	-	-	-
Acanthophora spicifera	80.86	1.84	159.70	12.48	14.84	3.91	-	5	-
Centroceras clavulatum	27.40	2.92	-	-	-	-	-	-	-
Gelidiella acerosa	8.99	60.80	11.79	26.33	-	-	-	20	-
Gracilaria parvispora	5.27	190.00	322.20	51.26	-	-	532.40	3	0.2
Gracilaria salicornia	17.46	60.82	46.97	97.25	17.77	-	-	4	-
Hypnea cervicornis	11.24	519.20	-	219.80	88.51	0.10	-	9	0.1
Kallymenia sessilis	24.56	-	-	-	-	-	-	24	-
Laurencia majuscula	58.39	51.29	-	-	-	-	-	-	-
Laurencia succisa	5.91	18.62	5.86	11.40	2.92	-	-	11	-
Plocamium sandvicense	-	94.41	-	-	-	-	-	30	-
Predaea weldii	23.22	3.99	-	-	-	-	-	31	-
Spyridia filamentosa	454.50	-	-	-	-	-	-	32	0.2
Tolypiocladia glomerulata	47.35	-	0.04	-	-	-	-	39	-
Trichogloea requienii	-	74.52	-	-	-	-	-	38	-
RHODOPHYTA-calcareous	-	3.42	-	-	-	-	-	51	-
Actinotrichia fragilis	4.31	6.13	-	-	-	-	-	14	-
Actinotrichia fragilis	-	118.20	21.64	-	-	-	-	35	-
Galaxaura rugosa	64.12	20.37	26.30	9.54	-	-	-	20	-
Hydrolithon reinboldii	5.81	5.64	-	0.67	11.92	-	-	17	-
Hydrolithon reinboldii	-	16.30	-	-	-	-	-	46	-
Liagora tetrasporifera	4.26	40.83	-	55.30	11.22	-	-	12	-
Porolithon gardineri	4.43	36.86	0.70	1.51	5.51	-	-	32	-
Porolithon gardineri									

Table IV. Concentrations of mycosporine-like amino acids in fleshy and calcareous brown algae.

Sample	Mycosporine-glycine	Shinorine	Porphyra-334	Palythine	Asterina-330	Palythinol	Palythene	Unknown 1 (% Total peak)	Unknown 2 (% Total peak)
	(nanamoles mg^{-1} protein)								
PHAEOPHYTA-fleshy									
Colpomenia sinuosa	160.90	0.21	-	-	-	-	-	14	-
Colpomenia sinuosa	6.79	-	-	-	-	-	-	12	-
Dictyopteris australis	-	-	-	-	-	-	-	100	-
Dictyopteris australis	131.50	4.51	7.09	-	-	-	-	14	4
Dictyota acutiloba	0.14	-	-	-	-	-	-	47	-
Dictyota acutiloba	3.98	0.75	-	-	-	-	-	26	-
Dictyota bartayresii	9.71	-	-	-	-	-	-	26	-
Dictyota sandvicensis	3.61	-	-	-	-	-	-	49	-
Hydroclathrus clathratus	1.14	-	-	-	-	-	-	19	-
Kappaphycus alvarezii	-	124.20	-	94.05	-	-	-	32	-
Kappaphycus alvarezii	1.33	47.53	1.08	46.42	12.33	-	-	16	-
Sargassum echinocarpus	-	2.18	-	-	-	-	-	22	-
Sargassum polyphyllum	-	-	-	-	-	-	-	46	-
Turbinaria ornata	-	-	-	-	-	-	-	38	-
Turbinaria ornata	-	-	-	-	-	-	-	47	-
PHAEOPHYTA-calcareous									
Padina japonica	13.92	1.45	0.12	-	-	-	-	15	-
Padina japonica	9.10	-	-	-	-	-	-	-	-

Table V. Concentrations of mycosporine-like amino acids in blue-green algae.

Sample	Mycosporine-glycine	Shinorine	Porphyra-334	Palythine	Asterina-330	Palythinol	Palythene	Unknown 1 (% Total peak)	Unknown 2 (% Total peak)
	(nanamoles mg^{-1} protein)								
CYANOPHYTA *Hormothamnion enteromorpholdes*	2.03	2.57	1.73	1.23	-	-	-	15	46
Lyngbya majuscula	-	2.92	-	-	27.17	-	-	6	3
Lyngbya sp.	-	-	-	-	-	2.11	-	54	-

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Effects of ultraviolet radiation and nitrogen enrichment on growth in the coral reef chlorophytes *Dictyosphaeria cavernosa* and *Dictyosphaeria versluysii*.

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ABSTRACT: Despite potentially harmful levels of solar ultraviolet radiation (UV), most coral reef acroalgae are restricted to shallow reef flats and few species are abundant on deeper reef slopes. For macroalgae on reef flats, exposure to UV may inhibit growth, as growth-limiting nutrients are directed to protection and repair pathways rather than to growth. For macroalgae on reef slopes, growth inhibition may decrease with decreasing UV exposure. In this study, the effects of UV exposure and nitrogen enrichment on growth were examined in two nitrogen-limited chlorophytes from Kane'ohe Bay, Hawai'i: the reef flat species *Dictyosphaeria versluysii*, and the reef slope species *Dictyosphaeria cavernosa*. Results from culture experiments indicated that ammonium enrichment enhanced growth rates in both species. At all nitrogen levels used, *D. cavernosa* growth rates were lower when exposed to UV-A and UV-B than when shielded from UV. Growth in *D. versluysii* was not affected by UV exposure at any nitrogen level, suggesting that UV defenses in this species are constitutive. Unlike some reef flat anthozoans with constitutive UV defenses, *D. versluysii* is not an obligate reef flat species in Kane'ohe Bay; transplant experiments indicated that changes in depth did not significantly affect growth rates. Nitrogen availability and grazing pressure appeared to have stronger effects on growth than did UV exposure, but variability in growth rate measurements may have masked some UV effects.

INTRODUCTION

Macroalgae are rarely abundant on pristine low-latitude coral reef slopes, but are often abundant on shallow coral reef flats (Gaines & Lubchenco, 1982; Hay, 1991). These distribution patterns have been attributed to higher grazing intensity on reef slopes than on reef flats, and to low productivity rates relative to grazing rates (Hatcher & Larkum, 1983; Hay, 1984a; Morrison, 1988). High levels of solar ultraviolet radiation (UV) make reef flats potentially stressful habitats, however (Jokiel, 1980). This raises the question of whether macroalgae found on reef flats are obligate or facultative reef flat dwellers (i. e., whether reef flats provide optimal conditions for productivity, or serve as refuges from herbivores but are suboptimal habitats). Obligate reef flat algae may be expected to minimize the costs of defenses to radiation, relative to productivity. In facultative reef flat algae, the relative costs of defenses are expected to be higher.

For any macroalga, synthesis of UV-protective agents and the repair or replacement of UV-damaged cell constituents are likely to incur metabolic costs. In nutrient-limited algae, nutrients may be allocated to UV protection or damage repair at the expense of growth or reproduction. Most metabolic pathways associated with UV exposure require nitrogen, eg., synthesis of UV-absorbing mycosporine-like amino acids (MAAs) and superoxide radical- and hydrogen peroxide-inactivating enzymes (Lesser & Schick, 1989; Lesser *et al.*, 1990), and resynthesis of damaged protein-pigment complexes (Bomman, 1989). For algae with broad depth distributions, the allocation of nitrogen to UV protection or damage repair may increase with increasing UV exposure, i.e., with decreasing depth. Growth rates of macroalgae restricted to shallow reef flats may be independent of UV exposure, either because high MAA levels are continuously maintained, or because metabolically inexpensive means of UV protection are used. Growth under varied conditions of UV exposure and nitrogen availability has not been examined in macroscopic algae.

Shallow water tropical and subtropical marine macroalgae from four taxonomic classes have been shown to contain MAAs (Sivalingam *et al.*, 1974; Wood, 1987, 1989; Post & Larkum, 1993). Increases in MAA concentration with increased UV exposure have been observed in zooxanthellate anthozoans (Jokiel & York, 1982; Dunlap *et al.*, 1986; Schick *et al.*, 1991; Kinzie, 1993) and macroalgae (Sivalingam *et al.*, 1974; Wood, 1987, 1989; Sherer *et al.*, 1988; Post & Larkum, 1993). These observations suggest that UV protection is induced and may be metabolically expensive. Exceptions to this pattern have been found among zooxanthellate anthozoans which are restricted to shallow water (Scelfo, 1985; Schick *et al.*, 1991; Stochaj *et al.*, 1994); MAA levels in these organisms appear to be constitutive rather than inducible.

In contrast to the narrow distributions of coral reef macroalgae described above, some algal species have become abundant on coral reef slopes near large human populations. These changes have been associated with reductions in herbivore densities and/or increases in nutrient loading (Hay, 1984b; Naim, 1993; Hughes, 1994; Littler *et al.*, 1994). Algal species which expand

their distributions down reef slopes under such conditions are clearly facultative reef flat dwellers. For reef flat species which do not invade reef slopes under the same conditions, the question raised above remains unanswered.

On the reef slopes of Kane'ohe Bay, O'ahu, Hawai'i, the chlorophyte *Dictyosphaeria cavernosa* has occupied large areas of reef slope for at least 30 years, overgrowing and displacing corals (Hunter & Evans, 1994). The success of *D. cavernosa* has been linked to anthropogenic nutrient enrichment (Smith *et al.*, 1981). Most macroalgal species in Kane'ohe Bay are restricted to the shallow flats of fringing and patch reefs, but *D. cavernosa* has a broad depth distribution, extending from outer reef flats down reef slopes to the muddy lagoon floor at 6-13 m depth (Hunter & Evans, 1994). This distribution spans a broad range of light penetration, as indicated by high extinction coefficients (approximately -0.23 · m^{-1} bay-wide in 1992, L. Watarai, unpublished data) relative to nearby oceanic water (approximately -0.07 · m^{-1}, Smith *et al.*, 1981). Solid substrata, required for attachment of thalli, rather than photosynthetically active radiation (PAR) appears to set the lower depth limit for this alga. Like other Kane'ohe Bay macroalgae, the congener *Dictyosphaeria versluysii* appears to be restricted to shallow reef flats and reef crests.

Dictyosphaeria cavernosa growth is strongly nitrogen-limited in Kane'ohe Bay; sustained inorganic nitrogen concentrations of ≥ 1 µM are required to maintain net growth (Stimson et al., in press). *D. cavernosa* growing under conditions of high UV and low nitrogen may therefore experience competition for nitrogen between UV protection and damage repair, and growth. As a result, the optimal physiological conditions for growth may be on reef slopes, below the depth of greatest UV exposure. *D. versluysii* growth is also nitrogen-limited in Kane'ohe Bay (Larned, in prep.).

In the present study, I tested the hypothesis that high levels of UV inhibit growth in *Dictyosphaeria cavernosa* and *D. versluysii*, and that inorganic nitrogen enrichment alleviates the effects of UV exposure. The narrow distribution of *D. versluysii* suggests that this alga may be an obligate reef flat dweller, and an alternative hypothesis is that UV protection in *D. versluysii* is constitutive, making the alga insensitive to changes in UV exposure. Before starting UV experiments, a field experiment was carried out to determine whether *D. versluysii* is a facultative or an obligate reef flat alga (i. e., whether its narrow distribution is associated with intense herbivory on reef slopes, or with physiological restrictions), and to measure potential and net growth in *D. versluysii* and *D. cavernosa* over a depth gradient.

MATERIALS AND METHODS

Field experiments

Thalli in herbivore exclusion cages and control thalli were used to measure potential and net growth rates over a range of depths on a reef slope from the reef crest to the lagoon floor. *Dictyosphaeria versluysii* thalli were collected from the windward reef flat at Coconut Island and allowed to acclimate in the field at 1 m depth for 4 days before the start of each experimental run. *D. cavernosa* thalli were collected at 1 m (reef flat), and 2, 4, 6 and 8 m below the reef flat on the slope of a patch reef near Coconut Island, transplanted within 1 hour to the same depths on the windward Coconut Island reef slope, and allowed to acclimate for 4 days. Acclimation periods were intended to minimize the effects of tissue damage during collection, and to reduce variability among initial thallus growth rates and tissue nutrient levels. Thalli were kept in flat cages (5 cm high) to prevent self-shading during acclimation periods.

Following acclimation, thalli of both species were grown at 5 depths on 1.5 cm mesh vinyl-coated metal trays both within and outside of 6 mm mesh herbivore exclusion cages. The 5 depths used were 1 (outer reef flat), 2, 4, 6 and 8 m below the outer reef flat at Coconut Island; the reef flat represents the approximate level of lower low tides, and the water depth over the reef flat at high tides is 0.5 - 1 m. Two thalli were attached to each tray outside the cage, and 1 or 2 thalli were attached inside the cage. Four to five replicate sets of caged and uncaged thalli were used at each depth. Specific growth rates (g · g^{-1} · d^{-1}) were calculated from initial and final fresh weights. Three runs of the experiment were carried out from May to August 1994. *Dictyosphaeria cavernosa* runs were 7 - 8 days long; *D. versluysii* runs were 12-20 days long to facilitate growth measurements in this slow-growing species.

Irradiance (PAR) was measured at the study site during the field experiment on 10 July 1994 with a submersible sensor and a 4 pi collector (Biospherical QSI-140). UV intensity was

measured by M. P. Lesser in spring of 1991 at approximately 1.5 m intervals from 0.15 to 7.6 m depth along a transect 10 m from the study site, using a spectroradiometer (Li-Cor LI-1800UW) and a cosine collector.

Factorial laboratory experiments

Laboratory experiments were carried out in an outdoor culture system to examine the effects of UV, ammonium enrichment, and interactions between UV and ammonium on the growth of *Dictyosphaeria cavernosa* and *D. versluysii*. Thalli were maintained in opaque white 3 liter containers, each holding 3 *D. cavernosa* thalli (range 1-3 g wet weight) or three *D. versluysii* thalli (range 0.75 - 1.5 g wet weight). Each container was supplied with flowing, 100 µm filtered seawater and aeration. Neutral density filters were used to reduce PAR to approximately 40% of full sun. Results from a preliminary laboratory experiment indicated that growth in *Dictyosphaeria cavernosa* is light-saturated at this irradiance level. Three levels of UV were provided with PAR-transparent filters: UV-opaque clear acrylic filters (UVO), UV-A-transparent Mylar® filters (UVA), and UV-A and -B-transparent Aclar® filters (UVT) (see Gulko *et al.*, this volume). Three levels of ammonium were provided: ambient seawater, ambient +2.5 µM, and ambient +5.0 µM. Ambient inorganic nitrogen levels during the experiment were 0.38 µM ammonium and 0.44 µM nitrate (N = 3 water samples). Each of the treatments was replicated twice for each species.

Thalli were continuously supplied with ambient or ammonium-enriched seawater during the experiment. A peristaltic pump was used to pump NH_4Cl solutions to nutrient-seawater mixing chambers upstream from the thalli containers. Fluorescein dye was used to check for complete mixing before the nutrient solutions reached the containers. Seawater flow rates (400 ml min^{-1}) were maintained with headboxes, and both seawater and nutrient concentrate flow rates were checked frequently. The culture system was cleaned every 3 days and the positions of treatments with respect to the headboxes were rotated to control for position effects. Thalli were cleaned and weighed every 3 days. Specific growth rates (g g^{-1} d^{-1}) based on fresh weights were calculated for each 3-day interval. Plots of growth rate vs. elapsed time confirmed that growth rates were fairly stable at the end of experimental runs.

Data analysis

For the laboratory experiments, the last set of growth increments calculated for each experimental run was used in analyses to minimize the effects of early acclimation to treatments on later growth rates. The mean growth rate of the three thalli in each culture container was treated as single data point. For the field experiments, growth rates were calculated using initial and final fresh weights. Factorial ANOVAs and Tukey multiple comparisons were used to compare differences among treatments. Where replicate experimental runs were carried out, runs were used as blocking variables.

RESULTS

Field experiments

Spectroradiometer measurements from spring of 1991 indicated that UV-A and UV-B (300 - 400 nm) was strongly absorbed in the first meter of seawater at the study site; the UV intensity at 1.6 m depth was 6.9 W m^{-2}, or 0.9% of the level just below the water surface. UV intensity was a decreasing logarithmic function of depth, and the relationship

$$\ln I_z = \ln I_0 - K(z),$$

where I_z and I_0 are the UV intensities at depth z and just below the surface, respectively, was used to calculate a UV extinction coefficient (linear regression coefficient of $\ln I_z$ on depth) of $K = -0.53 \cdot m^{-1}$ ($R^2 = 0.85$). PAR was measured on a single date (1200 hr, 10 July 1994) at the water surface above the study site, at the reef crest (0.5 m) and at the 5 depths used for the experiments. PAR was a decreasing logarithmic function of depth, ranging from 2023 µmol

quanta · m^{-2} · s^{-1} at the surface to 293 µmol quanta · m^{-2} · s^{-1} (14% of surface) at 8 m. The attenuation coefficient, calculated as for UV intensity, was -0.38 · m^{-1} (R^2 = 0.68).

The growth of caged *Dictyosphaeria cavernosa* thalli decreased with increasing depth while the growth of uncaged thalli showed no clear pattern; growth rates within all treatments were variable (Fig. 1). There were significant main effects of depth and caging on growth rates, but the interaction between caging and depth was not significant (Table I). Comparisons of growth rates among depths indicated that thalli at 1 m depth grew significantly faster than those below 2 m (Tukey test, $0.025 > p > 0.05$).

Table I. Factorial analysis of variance of *Dictyosphaeria cavernosa* growth rates in the field experiment. Cage levels: caged and uncaged. Depth levels: 1, 2, 4, 6, and 8 m.

SOURCE	DF	SS	MS	F	P
Cage	1	$6.03 \cdot 10^{-3}$	$6.03 \cdot 10^{-3}$	37.54	< 0.001
Depth	4	$1.72 \cdot 10^{-3}$	$4.31 \cdot 10^{-4}$	2.68	0.025 < P < 0.05
Cage X Depth	4	$1.47 \cdot 10^{-3}$	$3.67 \cdot 10^{-4}$	2.28	> 0.05
Block	2	$8.01 \cdot 10^{-4}$	$4.00 \cdot 10^{-4}$	2.49	> 0.05
Error	118	$1.90 \cdot 10^{-2}$	$1.61 \cdot 10^{-4}$		
Total	129	$2.90 \cdot 10^{-2}$			

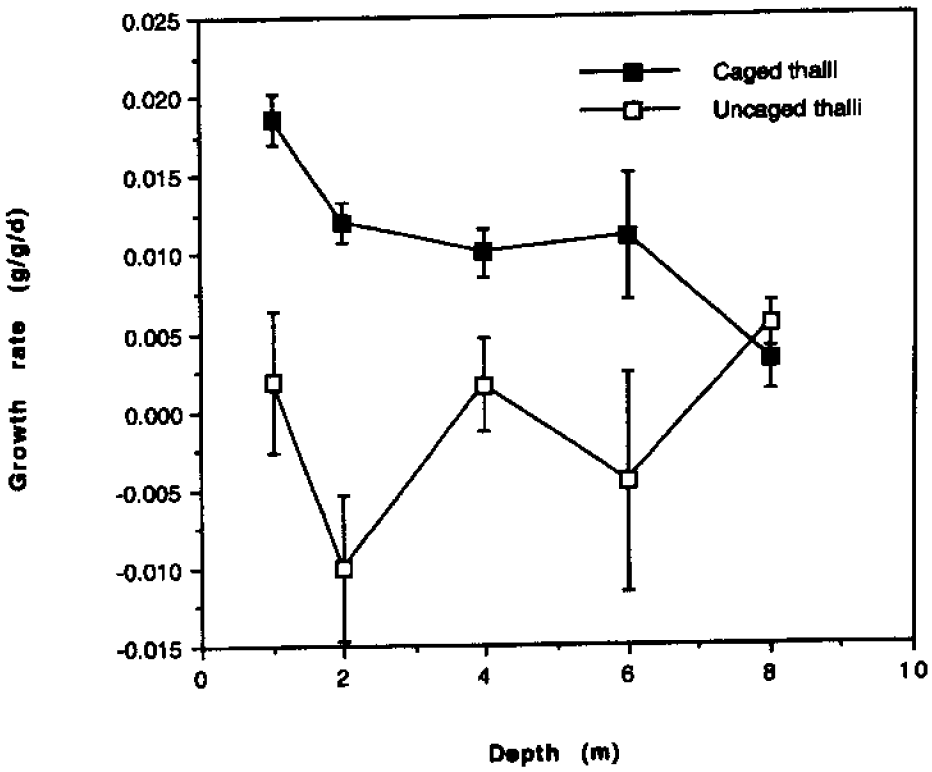

Figure 1. Growth rates ($g \cdot g^{-1} \cdot d^{-1}$) of *Dictyosphaeria cavernosa* thalli in the field experiment. Values shown are means of 3 runs, with 4 - 5 replicated treatments per run. Error bars are standard errors.

As with *Dictyosphaeria cavernosa*, the variability of *D. versluysii* growth rates within treatments was high (Fig. 2). Growth rates of caged *D. versluysii* thalli were significantly higher than those of uncaged thalli, but there was no effect of depth on growth rate, and the interaction between caging and depth was not significant (Table II).

Depth-averaged rates of tissue loss to herbivory (mean of all uncaged treatments) were nearly equal for both species: 0.17% d^{-1} in *Dictyosphaeria cavernosa* and 0.11% d^{-1} in *D. versluysii*. However, the depth-averaged potential growth rate (mean of all caged treatments) of *D. cavernosa* (1.19% d^{-1}) was much higher than in *D. versluysii* (0.02% $\cdot d^{-1}$). At 1 m depth, where the ranges of the two species overlap, the potential growth rate of *D. cavernosa* was 17 times that of *D. versluysii*; net *D. cavernosa* growth rate at 1 m depth was 7 times that of *D. versluysii* (Figs. 1 and 2).

Table II. Factorial analysis of variance of *Dictyosphaeria versluysii* growth rates in the field experiment. Cage levels: caged and uncaged. Depth levels: 1, 2, 4, 6, and 8 m.

SOURCE	DF	SS	MS	F	P
Cage	1	$1.12 \cdot 10^{-4}$	$1.12 \cdot 10^{-4}$	9.47	$0.001 < P < 0.005$
Depth	4	$6.88 \cdot 10^{-6}$	$1.72 \cdot 10^{-6}$	0.15	> 0.10
Cage X Depth	4	$6.77 \cdot 10^{-5}$	$1.69 \cdot 10^{-5}$	1.43	> 0.10
Block	2	$7.03 \cdot 10^{-4}$	$3.51 \cdot 10^{-4}$	29.65	< 0.001
Error	138	$1.64 \cdot 10^{-3}$	$1.19 \cdot 10^{-5}$		
Total	149	$2.52 \cdot 10^{-3}$			

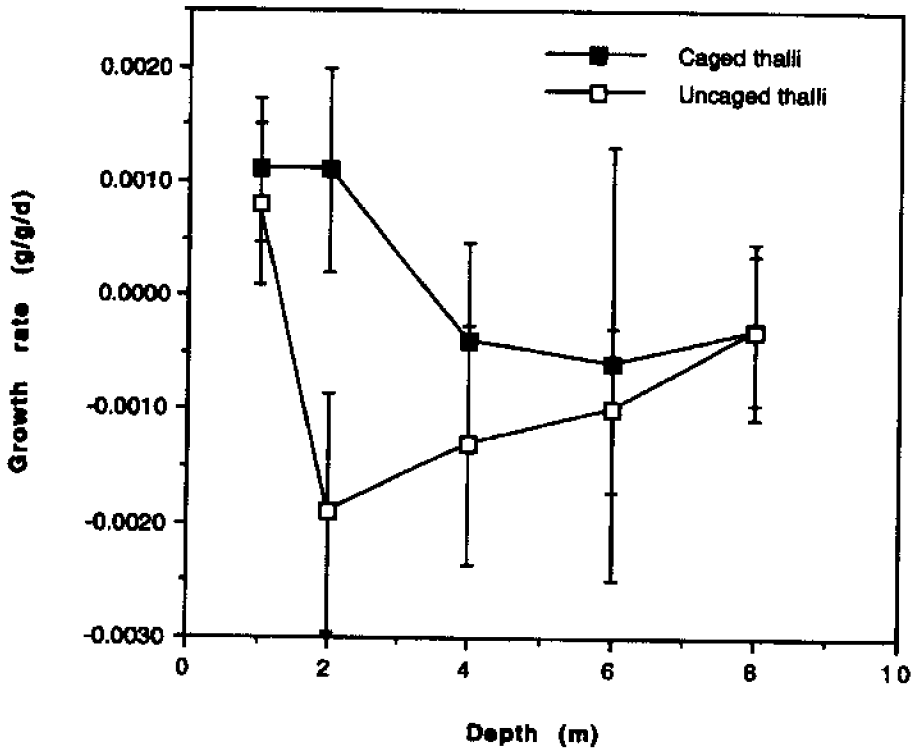

Figure 2. Growth rates (g g^{-1} d^{-1}) of *Dictyosphaeria versluysii* thalli in the field experiment. Values shown are means of 3 runs, with 5 replicated treatments per run. Error bars are standard errors.

Factorial laboratory experiments

Dictyosphaeria cavernosa growth rates in ammonium-enriched seawater were nearly 10 times those in unenriched seawater (Fig. 3), confirming that growth in this species is nitrogen-limited in Kane'ohe Bay. Both ammonium enrichment and UV level had significant effects on growth rate (Table III). There was no ammonium by UV interaction, however, indicating that the effects of one factor were not inhibited or enhanced by the other factor. Growth rates in both the ambient +2.5 μM and the ambient +5 μM ammonium treatments were significantly higher than the ambient treatments (Tukey tests, $p < 0.001$ for each comparison); differences between the two

enrichment treatments were not significant. Growth rates in the UVO treatments were significantly higher than in the UVT treatments ($0.025 < p < 0.05$); no other significant differences were detected among UV treatments.

Table III. Factorial analysis of variance of growth rates (g g^{-1} d^{-1}) of *Dictyosphaeria cavernosa* thalli in the ammonium enrichment by UV level experiment.

SOURCE	DF	SS	MS	F	P
NH4	2	0.00279	0.00139	42.3	< 0.001
UV	2	0.00039	0.00015	4.61	0.025 < P < 0.05
NH4 X UV	4	0.00011	0.000028	0.85	> 0.10
Error	9	0.00030	0.000033		
Total	17	0.00350			

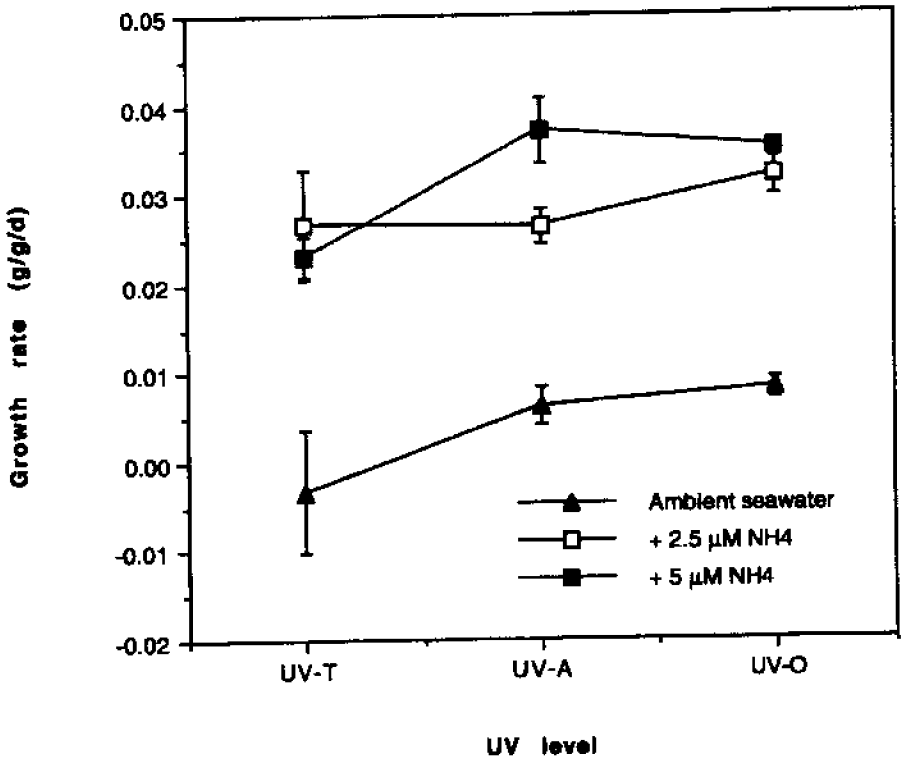

Figure 3. Growth rates (g g^{-1} d^{-1}) of *Dictyosphaeria cavernosa* thalli in the ammonium enrichment by UV laboratory experiment. Growth rates shown are for the final 2 days of the experiment and are means of two replicated treatments. Error bars are standard errors.

Growth rates of *Dictyosphaeria versluysii* in ammonium-enriched seawater were 2.5-4.5 times those in unenriched seawater (Fig. 4), indicating that, like *D. cavernosa*, *D. versluysii* growth is nitrogen-limited. Variability of growth rates within treatments was greater in *D. versluysii* than in *D. cavernosa*. Ammonium enrichment significantly affected *D. versluysii* growth

rates, but there was no significant effect of UV level, and no ammonium by UV interaction (Table IV). Comparisons of treatment means indicated that growth rates in the 2.5 µM ammonium treatments were significantly higher than the ambient treatments (Tukey test, $0.001 < p < 0.005$); no other significant differences were detected.

Table IV. Factorial analysis of variance of growth rates (g g^{-1} d^{-1}) of *Dictyosphaeria versluysii* thalli in the ammonium enrichment by UV level experiment.

SOURCE	DF	SS	MS	F	P
NH4	2	$3.65 \cdot 10^{-5}$	$1.83 \cdot 10^{-5}$	5.25	$0.025 < P < 0.05$
UV	2	$2.92 \cdot 10^{-6}$	$1.46 \cdot 10^{-6}$	0.42	> 0.10
NH4 X NO3	4	$1.87 \cdot 10^{-5}$	$4.70 \cdot 10^{-6}$	1.34	> 0.10
Error	9	$3.13 \cdot 10^{-5}$	$3.48 \cdot 10^{-6}$		
Total	17	$8.94 \cdot 10^{-5}$			

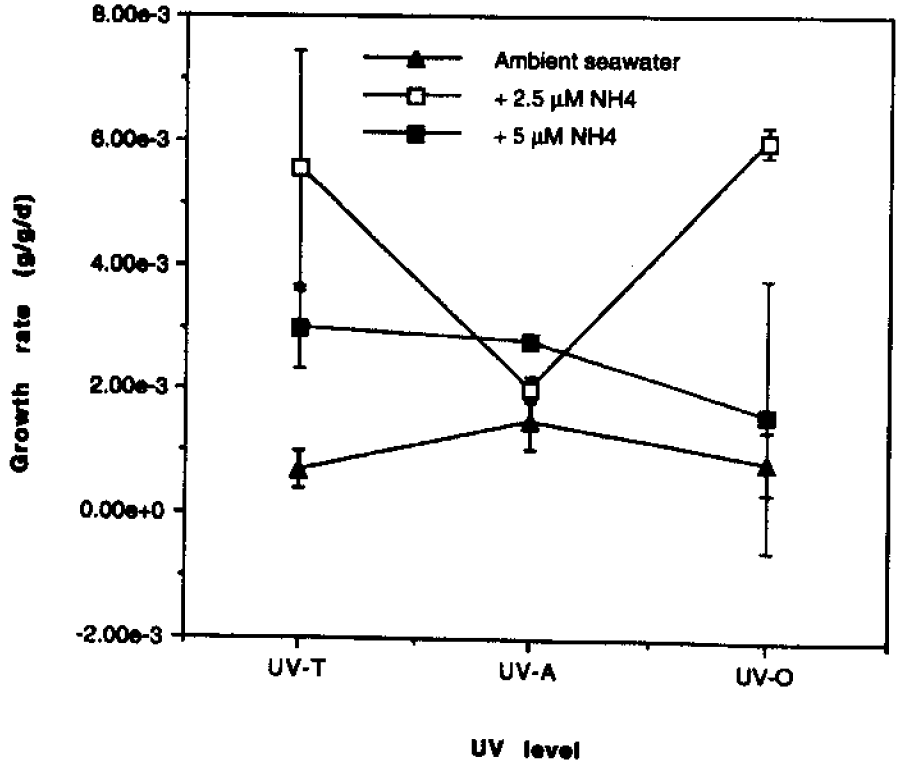

Figure 4. Growth rates (g g^{-1} d^{-1}) of *Dictyosphaeria versluysii* thalli in the ammonium enrichment by UV laboratory experiment. Growth rates shown are for the final 2 days of the experiment and are means of two replicated treatments. Error bars are standard errors.

DISCUSSION

Results of the field experiment suggest that herbivory and, on the upper reef slope (1 - 3 m), depth affect the growth of *Dictyosphaeria cavernosa*. Although net growth was very low between 1 and 6 m depth, this is a zone of high *D. cavernosa* cover on many Kane'ohe Bay reef slopes. The study site has no macroalgae within about 50 m to either side, however, and grazing intensity was probably higher there than at sites with abundant macroalgae. The interaction between caging and depth was not significant, but the sharp decline in growth rate at 2 m depth is probably due to severe grazing losses (Fig. 1).

Growth in *Dictyosphaeria cavernosa* showed a negative relation to UV level in the laboratory experiment. At all nitrogen levels, growth rates were lower in UVT treatments than in UVO treatments. The hypothesis that nitrogen enrichment alleviates the growth-suppressing effects of UV exposure was not supported. Rather, the difference between growth rates in UVO and UVT treatments was similar in all ammonium treatments (6.5-11 10^{-3} g g^{-1} d^{-1}).

Results of the laboratory experiment with *Dictyosphaeria versluysii* suggest that growth in this species is unaffected by the level of UV exposure. However, the hypothesis that insensitivity to UV is associated with physiological restriction to shallow, UV-rich environments was not supported. Protection from herbivory resulted in enhanced *D. versluysii* growth, but changes in depth did not significantly affect growth rates. Apparently, *D. versluysii* is not an obligate reef flat dweller in Kane'ohe Bay, but may be restricted to reef flats by high grazing pressure on reef slopes. *D. versluysii* has been collected in offshore Hawaiian waters as deep as 110 m (F. Norris, pers. comm.), indicating that the species is not physiologically restricted to shallow waters outside of Kane'ohe Bay. The observations that growth in *D. versluysii* is nitrogen-limited, and that net growth in the field was not sustained below 2 m depth, independent of caging, suggests an alternative hypothesis: *D. versluysii* may be restricted to reef flats by a higher availability of dissolved inorganic nitrogen (DIN) there. Water column DIN concentrations are generally higher over the reef flat near the study site than over the reef slope near the study site (reef flat DIN: approximately 1.3 µM; reef slope DIN: 0.5 µM) (Snidvongs & Kinzie, 1994; Stimson *et al.*, in press). In addition to elevated nutrient concentrations, rates of advection of nutrients may be more rapid across shallow reef flats than deeper reef slopes.

Although growth is nitrogen-limited in *Dictyosphaeria cavernosa* as well as in *D. versluysii*, *D. cavernosa* is not restricted to reef flats, and thalli growing on the reef slope may therefore have a means of acquiring nitrogen not shared with *D. versluysii*. Stimson *et al.* (in press) suggested that ammonium excreted by invertebrate epifauna on *D. cavernosa* thalli, and regenerated nitrate and ammonium from sediment patches isolated by thalli provide nitrogen subsidies, which allow *D. cavernosa* to grow and persist on reef slopes with low water column nutrient levels.

The low level of replication in this study and/or the variability inherent in growth rate measurements resulted in large error terms, and may have precluded the detection of some significant differences among treatments. Tissue growth is a composite process which integrates a large number of component processes (eg., photochemical reactions, carbon and nitrogen fixation, amino acid and protein synthesis), and changes in rates of component processes may confound experimental treatment effects on growth. In a study of the effects of UV exposure on the introduced rhodophyte *Kappaphycus striatum* in Kane'ohe Bay, Wood (1989) did not detect an effect of UV exposure on thallus growth, but did measure significant reductions in chlorophyll *a* and carotenoid concentrations, and increases in MAA concentrations in UV-exposed thalli. UV-B exposure has also been shown to increase respiration and reduce photosynthetic rate (oxygen evolution rate) in the macroscopic chlorophyte *Prasiola crispa* in Antarctica (Post & Larkum, 1993). Measurement of a variable which is a component of growth, and upon which both UV level and nitrogen availability have direct effects may provide more insight into the relationship between UV and nitrogen than does tissue growth rate.

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Phototoxicity in a coral reef flat community

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ABSTRACT: The synergistic effect of polycyclic aromatic hydrocarbons (PAHs) and ultraviolet radiation (UVR) was tested on coral reef flat organisms. Organisms from 5 animal phyla (Crustacea, Annelida, Mollusca, Porifera, and Cnidaria) were incubated in solutions of the PAHs, anthracene or pyrene, at concentrations below the saturation of these compounds in seawater, followed by exposure to UVR. Crustaceans, polychaetes and some cnidarians were affected at PAH concentrations and light intensities that can occur in their reef flat habitats . These concentrations of PAHs did not cause mortality in the absence of UVR and UVR did not cause mortality in the absence of PAH. PAH-induced phototoxicity of marine organisms was dependent on the concentration of PAH, intensity and wavelength of UVR, and taxonomic affinity; and occurred at PAH concentrations 3 to 4 orders of magnitude below that reported for toxicity by PAH alone. PAH-induced phototoxicity could be enhancing the general degradation of marine communities near urban areas. These effects may be exacerbated by increases in UVR due to ozone depletion. Direct mortality of certain species and age classes may have unexpected effects on reef communities. The sublethal effects are also unknown.

INTRODUCTION

Phototoxicity refers to the harmful effects of solar radiation on organisms. These effects can be the direct result of sunlight on the tissues of an organism (eg. sunburn) or they can be chemically mediated. Written records of the sun's ability to enhance the toxicity of certain chemicals (indirect phototoxicity) go back over 3000 years. Coal tar and anthracene were shown in the 1890s to cause blisters on human skin, upon later exposure to sunlight, and to promote skin cancer. Reports of indirect phototoxicity to aquatic organisms are more novel. Anthracene phototoxicity was first observed in freshwater fish by Bowling *et al.* (1983). Subsequent research on a variety of freshwater organisms, using anthracene and other polycyclic aromatic hydrocarbons (PAHs), have demonstrated that phototoxicity affects a variety of freshwater organisms (Oris *et al.*, 1984; Allred & Geisy, 1985; Oris & Giesy 1985, 1986, 1987; Kagan *et al.*, 1987; Oris *et al.*, 1990; McCloskey & Oris, 1991). There have been no reports of phototoxic effects of PAHs on marine organisms except for the brine shrimp, *Artemia salina* (Morgan & Warshawsky, 1977; Kagan *et al*, 1987). Results of freshwater studies indicate that phototoxic effects are dependent on PAH concentration in the aquatic environment, UVR wavelength (Ortis & Giesy, 1986), and intensity of UVR (Oris & Giesy, 1985). Previous studies in freshwater also determined that the synergistic effects of light and PAHs induce acute toxicity in organisms at concentrations hundreds to thousands of times below those reported for PAH alone (Oris *et al.*, 1984).

PAHs are chemicals composed of 2 or more fused benzene rings and are an important and ubiquitous class of pollutant chemicals in the environment. PAHs do occur naturally, but their concentrations in aquatic sediments are closely tied to anthropogenic fossil fuel utilization (Hites *et al.*, 1980). PAHs are produced by many processes including incineration of industrial and domestic wastes and the generation of power from fossil fuels (Neff, 1979); major sources of PAHs in the marine coastal environment are municipal waste, urban runoff, atmospheric precipitation, and petroleum spills (NRC, 1985). Waters in close proximity to large human populations have high PAH content but these compounds are not distributed uniformly: sediments have the highest concentrations, intermediate concentrations occur in aquatic organisms, and the lowest concentrations are found in the water column (Neff, 1979). Experimental evidence indicates that acute toxicity of PAHs to marine organisms occurs only at concentrations several orders of magnitude higher than those found in the most heavily polluted marine waters (Neff, 1979). Although acute toxicity to marine organisms occurs at high concentrations, relative to those found in the environment, PAHs are carcinogenic and chronic exposure poses a risk to the health of aquatic organisms (Neff, 1979).

The reef flat community in Kane'ohe Bay, Hawai'i is exposed to large amounts of urban runoff, especially in the south basin which supports a human population of more than 60,000 people (US

Department of Commerce, 1993). PAHs in urban runoff are derived from vehicle drippings and exhaust condensate, asphalt wear and leaching, and wear of vehicle tires (Neff, 1979). An additional source of PAHs to the south basin is sewage effluent. PAHs are produced by cooking and washing, and are contained in urine and feces. Historically, the south basin was subjected to large inputs of sewage effluent from circa 1935 to 1978 (Maragos, 1972), and although sewage is now directed to a deep-water outfall, PAHs incorporated into anoxic sediments may persist on a geologic time scale (Neff, 1979). Also, during periods of heavy rain fall, sewage spills into the south basin of Kaneohe Bay, a release compounded by PAHs entering the bay in runoff from channelized streams. Concentrations of PAHs in water samples increases significantly after heavy rainfall and reports of PAH concentrations in the µg/l (ppb) range are common for marine waters near urban areas (Neff, 1979).

Tropical reef flat organisms also are exposed to high doses of solar UVR due to the transparent nature of tropical waters (Jerlov, 1968; Kufner, 1995) and the shallow depths characteristic of this habitat. Reef flats are generally covered by less than 2 m of water and are aerially exposed during neap low tides. Our investigation was prompted by the lack of information on the phototoxic effects of PAHs on marine organisms and the certain exposure to PAHs and solar UVR that reef flat organisms must experience. We posed the following questions: 1) Are coral reef flat organisms sensitive to PAH-induced phototoxicity, 2) does the phototoxic response depend on the intensity or wavelength of UVR, and 3) is the intensity of ambient solar radiation adequate to induce phototoxicity in reef flat organisms?

MATERIALS AND METHODS

Phototoxicity Experiments

The phototoxic effects of the PAHs, anthracene and pyrene, were tested on representative species from the coral reef flat community. These preliminary experiments were simply designed to determine if reef flat organisms are sensitive to PAH-induced phototoxicity. Laboratory experiments using an artificial light source were performed with organisms collected from wild populations. The collection site was along the east side of Coconut Island located in Kane'ohe Bay, O'ahu, Hawai'i. During experiments, larger-sized organisms were contained in 50-250 ml beakers and larvae were held in depressions on porcelain culture plates.

Stock solutions contained 0.2 g/l anthracene or pyrene (Aldrich Chemical Co.) in methanol. A series of PAH concentrations was prepared by injecting aliquots of stock solution below the water surface with a microsyringe and 0, 16, 32, 48 µg/l PAH. The solubility of anthracene in seawater (33 $^{o}/_{oo}$, 25° C), 32 µg/l (Whitehouse, 1984), is considerably lower than that of pyrene ($35^{o}/_{oo}$, 25° C) 90 µg/l (Rossi, 1981). The highest concentration of anthracene (48 µg/l) probably was above it's saturation solubility in seawater but was included for comparisons of toxicity between anthracene and pyrene; the highest concentration of pyrene was well below its saturation point. To control for possible toxic effects of methanol, beakers containing 0 µg/l PAH were injected with the amount of methanol used to carry PAH into the highest concentration in the series. Each experiment consisted of three sets of each concentration series, two of which were exposed to UVR (Series A and Series B) and one was shielded from the light source (Control Series) with aluminum foil. Experiments were placed into waterbaths to maintain temperature at that of ambient seawater (24 - 26° C). Organisms were incubated in the seawater/PAH solution for 2 hr before exposure to UVR, the source of which was a GE™ F40 Blacklight positioned 4.5 cm above the platform. During exposure to UVR the organisms were observed every hour for up to 8 hr. UVR intensity was measured with a Spectroline Model DM-365N light meter and ranged between 975 to 1000 µW cm^{-2}. Motile animals that did not respond to gentle prodding were considered dead. For non-motile organisms other criteria were used to distinguish affected organisms, such as, discoloration of sponges and bleaching of corals.

UV Wavelength Experiment

Anthracene and pyrene, show absorption peaks in the UV portion of the electromagnetic spectrum. Light sources with peak output in regions coinciding with UV absorption by anthracene (GE' F40 Blacklight) and pyrene (Westinghouse™ Sunlamp FS20) were selected to test the hypothesis that phototoxicity is dependent on wavelength of radiation available. The absorption spectrum of anthracene peaks in the long wavelength UVR region (335 - 375 nm), whereas that of pyrene peaks at shorter wavelengths (310 - 335 nm). *Artemia salina* were selected for this experiment because they have been shown to be susceptible to phototoxicity in previous studies (Morgan & Warshawsky, 1977; Kagan *et al.*, 1987). One day old *A. salina* were incubated for 2 h with anthracene or pyrene, irradiated with the long-wavelength UV light source, and observed after 1 h. Another set of *A. salina* was incubated in anthracene or pyrene and then irradiated with the short-wavelength source for comparison.

UV Intensity Experiment

The effects of UV intensity on phototoxicity were investigated using the same protocol described above, except that exposure to UV from an artificial source was replaced by exposure to solar UV. The intensity of ambient solar UVR during experiments ranged between 407 $\mu W\ cm^{-2}$ under cloud cover to 1428 $\mu W\ cm^{-2}$ under clear skies. To test the effects of radiation intensity, one day old *Artemia salina* were treated to the same concentration series of pyrene or anthracene used in above experiments and then exposed to ambient solar radiation. The control series was covered with acrylic (Plexiglas™ G UF-3) that is transparent to the visible spectrum of light but is opaque to UVR.

Phototoxicity in Ambient Solar Radiation

Experiments with a variety of organisms were conducted using the same series of PAH concentrations and ambient solar radiation to investigate phototoxicity in a light regime more closely approximating field conditions. These organisms included branch tips of the corals *Montipora verrucosa*, *Porites compressa*, and *Pocillopora damicornus*, and larvae of *Fungia scutaria*. Other organisms tested included the anthozoan *Zoanthus pacificus*, the sponge *Callispongia diffusa*, the polychaete *Platynereis dumerilii* and the gastropod *Bittium parcum*. The crustacean species included zoea larvae of the alpheid shrimp *Alpheopsis* sp., and the amphipod *Amphilocus likelike*. Beakers with larger organisms were wrapped with aluminum foil and larvae, held in porcelain culture dishes, were covered with UV-opaque acrylic to shield the control series from UVR.

RESULTS

Phototoxicity Experiments

Branch tips of the corals *Montipora verrucosa* and *Pocillopora damicornis*, and the gastropod *Bittium parcum*, showed no observable phototoxic effect with pyrene and UVR. Larvae of the unidentified black sponge had 100% mortality in all treatments exposed to UV, indicating a direct phototoxic effect of UV on these larvae. PAH-induced phototoxicity was observed with the amphipod *Amphilocus likelike*. The percent mortality increased with increasing concentrations of pyrene (Fig. 1), while no mortality was observed in the controls. The percent mortality of the polychaete *Platynereis dumerilii* increased dramatically at the highest concentration of pyrene ($48 \mu g\ liter^{-1}$) and ranged from 90 to 100 percent (Fig. 2) with no mortality in the controls.

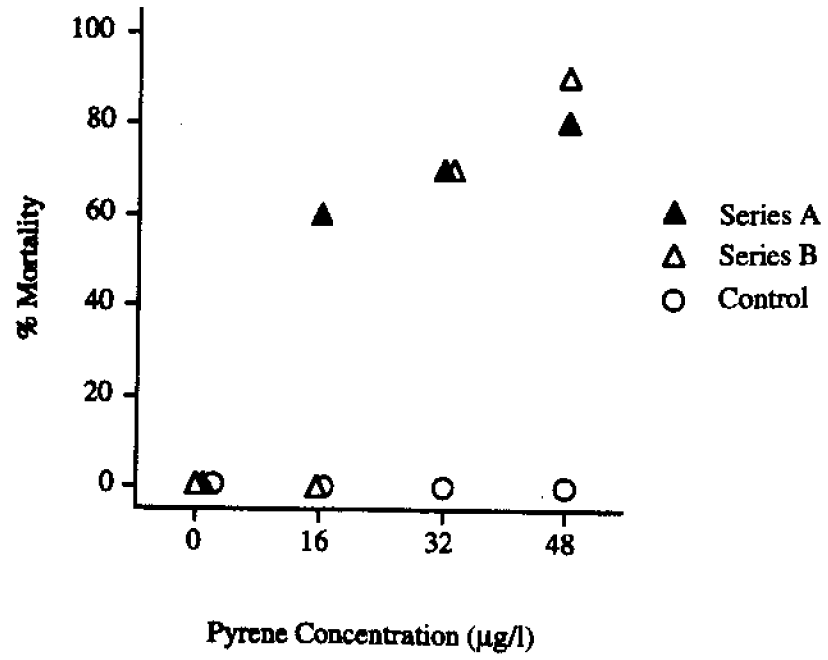

Figure 1. Effect of pyrene and UVR on the amphipod, *Amphilocus tkalike*. Amphipods in the pyrene concentration Series A and Series B were exposed to UVR, amphipods in the Control Series were shielded from UVR.

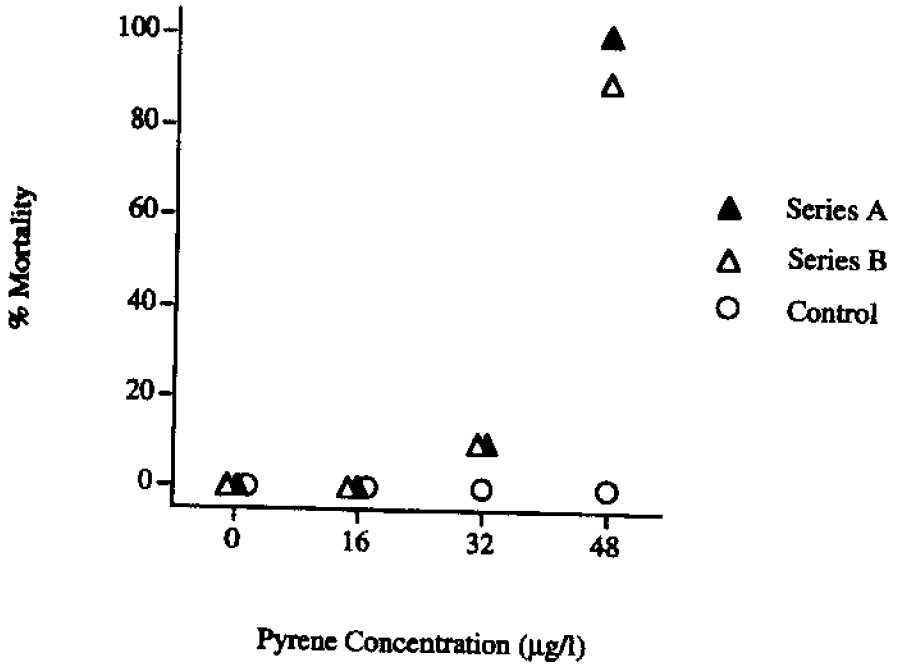

Figure 2. Effect of pyrene and UVR on the polychaete *Platynereis dumerilii*. Polychaetes in the pyrene concentration Series A and Series B were exposed to UVR; polychaetes in the Control Series were shielded from UVR.

UV Wavelength Experiment

Artemia salina incubated in anthracene (which has peak absorption in the long-wavelength UV), showed higher mortality when exposed to the long-wavelength UV light source than those exposed to the short-wavelength UV light source (Fig. 3). *A. salina* incubated in pyrene (which has peak absorption in the short-wave length UV), showed higher mortality when exposed to the short-wavelength UV source than when exposed to the long-wavelength UV source. There was some mortality of *A. salina* controls during these experiments, but the overall mortality rate was very low. These results support the hypothesis that toxicity is wavelength-specific and coincides with the peak in the absorption spectrum of the PAH utilized.

UV Intensity Experiment

Mortality of brine shrimp was 100 percent in the upper concentration of anthracene or pyrene within 1 hr after exposure to ambient solar UVR (Fig. 4). At the same concentrations of PAH in the laboratory mortality ranged between 0 and 70 percent (Fig. 3). These results support the hypothesis that phototoxicity depends on light intensity which overall was greater outside on a partly cloudy day than in the laboratory under lamps. As the intensity of outdoor light varied greatly, the actual amount of time for mortality to occur at a particular light intensity is unknown.

Phototoxicity in Ambient Solar UV

Callispongia diffusa, a purple tubular sponge which is common on the reef flat, showed no response to PAH under ambient solar UV. Similar results were obtained with the black gastropod *Bittium parcum* and the zoanthid *Zoanthus pacificus*. Branch tips of the corals *Montipora verrucosa*, *Pocillopora damicornis* and *Porites compressa* showed various responses to pyrene in ambient solar UV. The day following treatment, *M. verrucosa* tips were bleached in the pyrene concentrations of 16, 32 and 48 µg/l in treatments exposed to UV; the control series appeared normal. Tips of *P. damicornis* were bleached only in the 48 µg/l concentrations exposed to UV and *P. compressa* tips appeared normal in all cases. Larvae of the alpheid shrimp, *Alpheopsis* sp., showed 100% mortality in anthracene or pyrene at concentrations of 32 and 48 µg/l within 1 h of exposure to UV. Larvae of the mushroom coral, *Fungia scutaria*, showed 100% mortality in the highest pyrene concentration within 1 h.

DISCUSSION

This is the first report of PAH-induced phototoxicity in tropical marine organisms. The phototoxic response depends on a combination of factors: 1) the concentration of PAH in the water, 2) the intensity and wavelength of UVR, and 3) the sensitivity of the organism. During these experiments, the authors were careful to keep the concentrations of chemical and the experimental light conditions within those that can occur in the reef flat environment. Acute toxicity was observed for some organisms at concentrations of PAH as low as 16 µg l^{-1} (ppb) under light conditions that were considerably less intense than field conditions. Results of these experiments suggest that phototoxicity may be occurring in natural systems and that organisms in tropical shallow water habitats are at high-risk due to the transparency of the water and the high dose of solar UV to which they are exposed.

Neff's (1979) compilation of acute toxicity of PAHs to freshwater and marine animals, shows times to achieve a 50% mortality rate to be on the order of 18 hr to >10 days. The time scale of our experiments was quite brief and acute toxicity to 100% of the organisms exposed to PAH and ambient solar radiation took less than 1 hr for brine shrimp, coral larvae and shrimp larvae. This demonstrates that these chemicals become extremely toxic when combined with UVR. As PAH alone requires concentrations on the order of mg l^{-1} (ppm) for toxicity, toxic concentrations on the order of µg l^{-1} (ppb) in our tests make the probability of harm even more compelling.

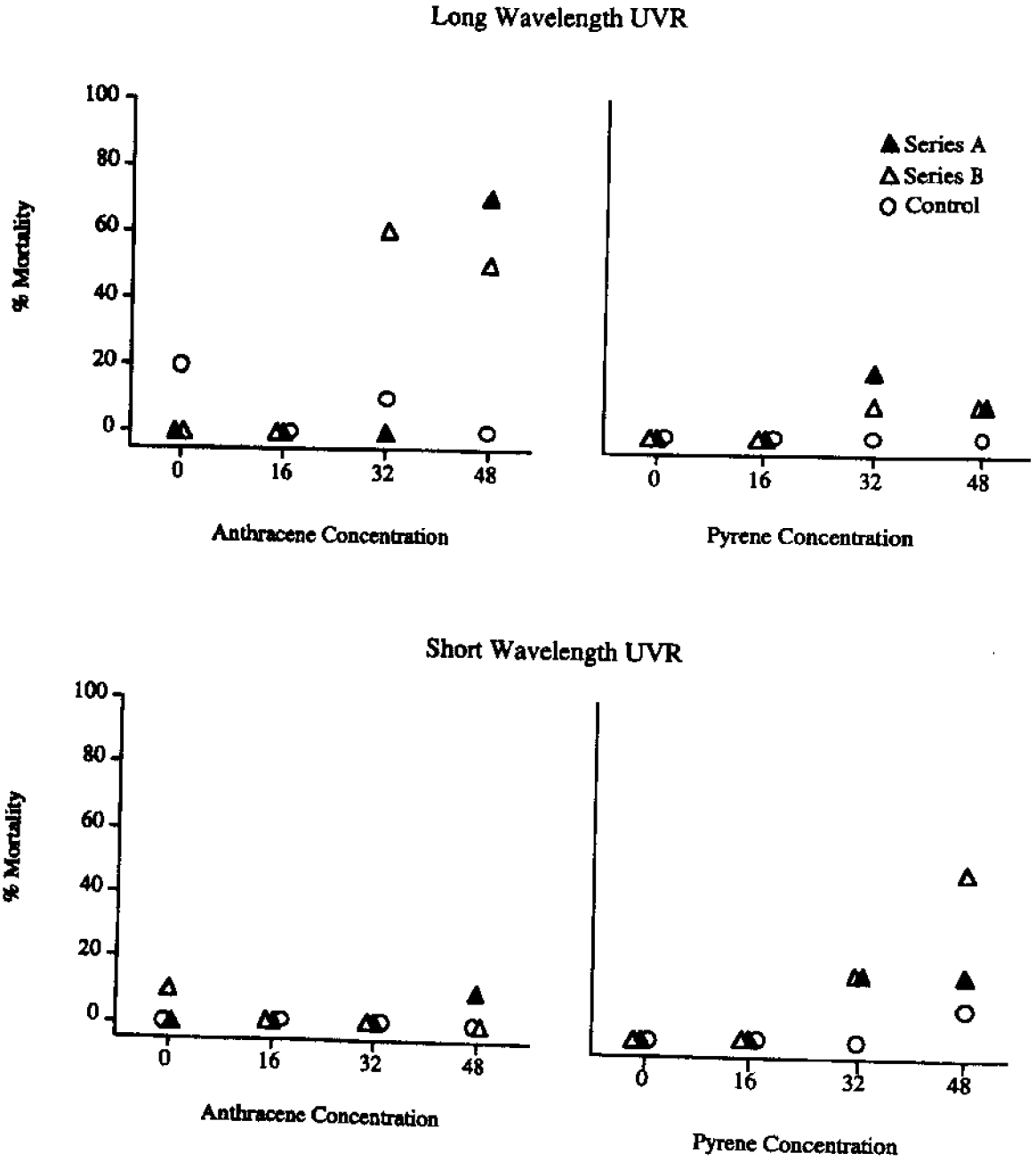

Figure 3. One day old *Artemia salina* larvae incubated in pyrene or anthracene, then exposed to long- or short-wavelength UVR. Each graph represents 3 PAH concentration series (mg/l) in which Series A and Series B were exposed to UVR and the Control Series was shielded from UVR.

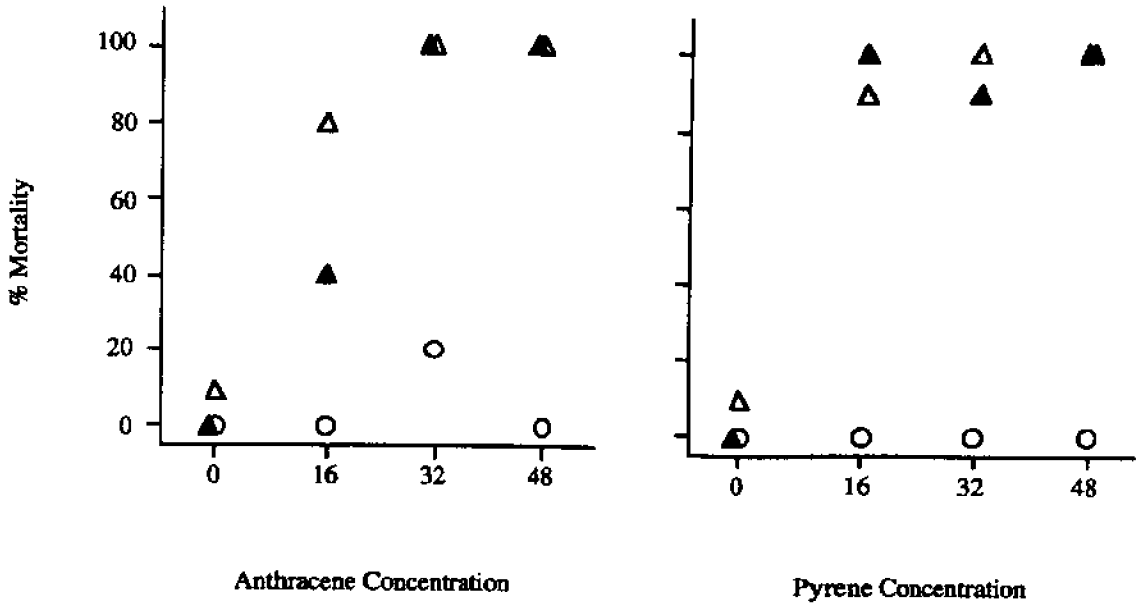

Figure 4. One day old *Artemia salina* larvae after incubation in pyrene or anthracene followed by exposure to ambient solar radiation. Each graph represents 3 PAH concentration series (mg/l) in which Series A and Series B were exposed to solar radiation and the Control series was shielded from the UV portion of the spectrum with acrylic.

The variation in sensitivity to phototoxicity of organisms in the same taxonomic class is remarkable. The corals exposed to ambient solar UVR are a case in point. The coral that is most common in the shallow waters of Kane'ohe Bay, *Porites compressa*, was not visibly affected by PAH at the concentrations used, while *Montipora verrucosa*, which is generally found at greater depths was bleached and *Pocillopora damicornis*, which is almost exclusively found on the reef flat also bleached. This difference might be associated with the natural UV blockers found in many corals (Dunlap and Chalker, 1986). Larvae of the mushroom coral, *Fungia scutaria*, were affected even in the lowest concentration of PAH, indicating that some larvae may be highly sensitive to phototoxicity.

Phototoxicity may have a greater affect on the developmental stages of organisms, especially those that spend their early life in shallow nearshore environments. High sensitivity of larvae could negatively impact coral populations of species that are not susceptible to phototoxicity as adults. Fish larvae also appear sensitive to phototoxicity. Freshwater fish that found shade in outdoor flume experiments were able to survive, while those exposed to full radiation were killed (Bowling et al., 1983). Habitats that provide shade, such as mangroves, may function as a refuge for fish and larvae that make their way to these areas. These relationships are fertile ground for future research.

The organisms that were not affected by phototoxicity in these experiments include sponges, gastropods, corals and anthozoans. Their resistance to phototoxicity may be a result of an ability to metabolize PAHs at a higher rate than other organisms, or the possession of UV blockers that absorb or diffuse UV energy; most resistant organisms were dark-colored. The ability to resist phototoxicity will favor the survival of resistant species in urbanized shallow water habitats and could change the composition and structure of marine communities in these areas. In fact, such changes may have already taken place.

Acute toxicity to marine organisms occurs at concentrations of PAH well below the saturation in seawater. Further investigation into this phenomenon may lead to regulation and treatment of runoff and sewage effluent to reduce the PAH content. Toxicologists have been concerned about PAHs in the aquatic environment for decades, but the sublethal effects obtained in past studies with PAHs did not compel managers to restrict these chemicals. The predicted increase in solar UVR due to ozone thinning may compound the difficulty in estimating safe levels of PAHs in the aquatic environment.

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UV vision by marine animals: mainly questions

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ABSTRACT: Ultraviolet (UV) light penetrates to considerable depths in the oceanic environment. Numerous marine species, both fish and invertebrates, are able to sense this UV radiation. Some of these species may use UV vision for detection of plankton on which they prey, and for orientation. In general, the information carried by UV light, the role of UV sensitivity, and its distribution among marine animals, are yet to be determined.

INTRODUCTION

"Animals have eyes for seeing, i.e., to discriminate between variations in the environmental light distribution" (Stavenga, 1984). In the ocean, distinct continuous changes in the photic environment (Loew & McFarland, 1990) present demanding conditions for these functions of visual systems. Changes in light intensity, due to the sun's movement, occur at a rate of 0.1% per minute at noon, but up to 50% per minute during twilight (McFarland, 1986). Clouds passing above the water decrease light intensity by up to 75% (McFarland, 1986), and focusing of light beams by surface waves, known as flicker, may double the illumination of specific objects and enhance their visual contrast (McFarland & Loew, 1983; Loew & McFarland, 1990).

In addition to changes in the ambient light field due to variations in incoming irradiance, light changes in intensity, spectrum, and polarization with depth and direction of view. Downwelling light differs from that which is upwelling, and light coming through Snell's window is distinct from that which is internally reflected down from the surface of the water.

The classification of light by wavelength into infrared ($\lambda > 700$ nm), visible (400-700 nm), and ultraviolet ($\lambda < 400$ nm), is based on the sensitivity of the human visual system. The "visible" range roughly corresponds to the wavelengths of photosynthetically active radiation (PAR). Although the 400 nm cutoff does not necessarily have any particular meaning to other animals, as humans we are attracted to the visual information obscure from our eyes.

UV light penetrates to considerable depths in tropical marine waters, and especially in the clear, oligotropic waters surrounding coral reefs (Jerlov, 1968; Tyler & Smith, 1967; Fleischman, 1989). McFarland (1986) calculated that in clear sea waters, a visual pigment system with an absorbance λ_{max} of 310 nm will be capable of photopic vision, at noon, down to 50 m, and down to 100 m if its sensitivity λ_{max} is 350 nm.

Since UV light is strongly absorbed by water, the upwelling light has a narrower spectral band width, containing less UV radiance than downwelling light (Tyler & Smith, 1967). Light reflected internally down from the water surface has a very different spectrum than that coming into the water column from the air, creating a region of high contrast at the edge of Snell's window. In the sea, this edge is not a sharp line but a region in which objects are alternately illuminated by the two sources, or are seen alternately against them.

These ever-changing conditions permit the development of visual systems, which either specialize for specific conditions or parts of the spectrum, or generalize for a broad band of environmental conditions (Lythgoe, 1979). UV vision is one of these visual mechanisms which provides the animals with information that is unavailable to humans.

FISH UV VISION

Numerous species of fresh water fish are sensitive to UV light (Browman *et al.*, 1994; Harosi & Hasimoto, 1983; Harosi, 1985; Jacobs, 1992; Robinson *et al.*, 1993). Several anadromous species, including species of salmonids, and the killifish *Fundulus hetreoclitus* (Harosi & Fukorotami, 1986) are capable of UV vision as well. In rainbow trout (*Onocorhynchus mykiss*), brown trout (*Salmo trutta*), and Atlantic salmon (*Salmo salar*), UV photoreceptors are limited to juvenile fish (Kunz *et al.*, 1994). Kunz *et al.*, (1994) reported that Atlantic salmon (*Salmo salar*) lose their UV sensitivity due to degradation of the photoreceptors in the fish retina. Only a single study (McFarland & Loew, 1994) has documented the existence of UV absorbing visual pigments in marine fishes. These are the tropical damselfish, *Dascyllus trimaculatus* and *Pomacentrus coelestis*, and the sub-temperate Blacksmith (*Chromis punctipinnis*), which are all planktivorous.

To date, except for the association with polarized light body orientation, the only function

recognized to be associated with fish UV vision is feeding on plankton. UV sensitivity is known to improve plankton capture by rainbow trout and pumpkinseed sunfish (Browman *et al.*, 1994) and juvenile yellow perch are able to feed under UV illumination (Loew *et al.*, 1993). Additionally, UV sensitive stages of salmonids are planktivores, while the adults prey on larger items.

UV sensitivity does not come cheaply to fish. UV radiation may damage the retina of the fish, especially those active during day time in shallow waters. Indeed, numerous marine teleosts have UV-absorbing pigments in their lenses (Dunlap *et al.*, 1989; Douglas & Thorpe, 1992; Thorpe *et al.*, 1993). These pigments are presumed to protect against UV damage (Dunlap *et al.*, 1989; Thorpe *et al.*, 1993), though their existence in deep sea fish suggests other roles as well (Douglas & Thorpe, 1992; Thorpe *et al.*, 1993). Some of these compounds have been identified as micosporine-like amino acids (MAAs) (Dunlap *et al.*, 1989; Thorpe *et al.*, 1993) which are associated with UV protection in other organisms. These pigments, each having a narrow absorption spectrum, when combined together can absorb light over the range of 320-395 nm.

INVERTEBRATE UV VISION

Among aquatic invertebrates, UV sensitivity has been described in a limited number of species, most of them arthropods. A single case of UV sensitivity is known among the molluscs, where the giant clam *Tridacna* has a receptor class with maximal response at 360 nm (Wilkens, 1984). Crustaceans which have photopigments with maximal absorbance in the UV range include the fresh water *Daphnia magna*- λ_{max} 348 nm (Smith & Macagno, 1990), and the common prawn *Palaemonetes vulgaris*- λ_{max} 390 nm (Wald & Seldin, 1968), the spiny lobster *Panulirus argus*- λ_{max} 370 nm (Cummins *et al.*, 1984), several species of mantis shrimp- λ_{max} 325-340 nm (Cronin *et al.*, 1994), and several species of deep sea crustaceans: *Janicella spinacauda*- λ_{max} 370 nm, *Oplophorus spinosus* and *Oplophorus gracilirostris*- λ_{max} 350 nm (Frank & Case, 1988), and *Systellaspis debilis* (Frank & Widder, 1994). The photoreceptors in the median eye of the xiphosuran *Limulus polyphemus* have a λ_{max} of 360 nm (Wald & Krainin, 1963), though the role of this median eye is not fully understood. In addition to this "true" UV sensitivity, some organisms such as crayfish (Cummins & Goldsmith, 1981) and crabs (Martin & Mote, 1982) have visual pigments with maximal absorbance in the violet-blue region. These pigments are expected to absorb and respond to near-UV light as well.

Several marine organisms show sensitivity to UV light, but do not use this sensitivity for "true vision", i.e. using light to image the external world. The salt water bacterium, *Halobacterium halobium*, displays antagonistic responses to UV illumination- λ_{max} 280 and 370 nm and to visible light- λ_{max} 360 nm, although the mechanism of this response is not well understood (reviewed by Menzel, 1979). The sea anemone, *Anthopleura xanthogrammica*, retracts its tentacles in response to UV illumination (λ_{max} 360 nm), but bends them in response to visible light (λ_{max} 500 nm) (Clark & Kimeldorf, 1970; Menzel, 1979).

UV VISION AND POLARIZATION SENSITIVITY

UV sensitivity is frequently coupled with sensitivity to partially linearly polarized light. Several fish species (Harosi, 1985; Hawryshyn, 1992) as well as mantis shrimps (Marshall *et al.*, 1991) use their UV photoreceptors to sense polarized light. This polarization sensitivity may be used for navigation, spatial orientation, and for detecting specific objects. The underwater light field is partially linearly polarized with the orientation of polarization depending on the time of day, the depth, the optical properties of the water, and the angle of view (Tyler, 1963). Shiny objects, such as fish, reflect light at specific orientations of polarization (Cameron & Pugh, 1991; Hawryshyn, 1992), and depolarizing organisms such as plankton may seem conspicuous against a partially linear polarized background (Loew, pers. comm.). However, the role of UV-based polarization sensitivity is as yet unknown.

UTILITY OF UV VISION

A simple reason for the existence of UV vision is to expand the spectral range over which animals can obtain visual information (Jacobs, 1992). However, the existence of UV-protecting compounds in the lenses of numerous fish suggests that maintaining UV vision may have some disadvantages, and therefore the benefits to the animals that preserve it should be considered.

The high scattering of UV light in sea water (Lythgoe, 1979) may create a bright

homogeneous background, mainly at the direction of Snell's window. Dark objects, such as plankton, and fish will be highlighted against such a background. Cronin *et al.*, (1994) demonstrated this phenomenon where a school of fish appear more noticeable when viewed against strongly scattered UV light in a horizontal direction, using a UV-transmitting (but visible) light-blocking filter. Zooplankton, which are exposed to high UV radiation, maintain UV-absorbing compounds, such as MAAs, that are believed to serve as "sun screens" and protect internal tissue from UV damage. These animals are transparent through the visual range but will be conspicuous when viewed in the range in which these compounds absorb (Loew, pers. comm.). On the other hand, the high scattering of UV light tends to blur the image and make a UV image-forming system less useful than one based on longer wavelengths (McFarland, 1986).

The interaction between UV receptors and one or more receptors in the human visual range (400-700 nm), may form a multichannel mechanism capable of breaking camouflage or detecting differences in the incoming spectrum. Such systems commonly function by looking at the difference, or contrast, between the inputs. The complex visual system of stomatapods, with their numerous visual pigments, may function in such a comparative way (Cronin & Marshall, 1989).

UV vision can be used to detect specific patterns which are unseen, or appear differently, in the visible range. Harosi and Hasimoto (1983) demonstrated that fresh water Japanese dace (*Tribolodon hakonensis*) have UV absorbing cones - λ_{max} 350 - 370 nm. The UV body coloration of these fish display strips which do not appear when viewed through a UV-blocking green filter (Harosi, 1985). It is reasonable to assume that other animals have UV-colored patterns as well. The role of such patterns in communication is yet to be established.

Sensitivity to UV illumination by deep-sea crustaceans presents a most intriguing problem: light at the depths in which they live (more than 500 m during the day) is very limited and is presumed to contain very limited amounts of UV light. Therefore, one may ask, "What use is there for UV sensitivity for animals living at these depths?" UV sensitivity may be used for discrimination between bioluminescence from different organisms (Frank & Case, 1988). As four species of deep sea crustaceans that are known to possess UV sensitivity display diurnal vertical migration, Frank and Widder (1994) suggested that UV sensitivity is part of a two-channel mechanism used to identify changes in the ambient light environment, set to trigger the vertical migration.

AN ON-GOING CHALLENGE

At the current level of knowledge, it seems that a discussion on UV vision in the marine environment presents more questions than answers.

McFarland & Loew (1994) presented a key difficulty which must be answered before any serious ecological discussion about marine UV vision can occur: we need to know which animals possess UV sensitivity. At present, the number of marine species examined for sensitivity to UV light is extremely small. However, it is likely that UV vision is present among coral reef fishes (McFarland, 1991) and other species exposed to UV radiation. Therefore, an effort needs to be made to examine more marine animal species, both fish and invertebrates, so that we can have a basis for comparison.

Along with looking at the animal's sensitivity, detailed measurements and imaging of the underwater UV light field are required so that we can start looking for specific information which is carried by UV light. Better understanding of this information will enable us to isolate tasks that can be performed through the use of UV vision. Behavioral studies can follow and examine whether these tasks are indeed performed.

The association of UV vision and polarization sensitivity is not clear either. What is the unique property of the short-end of the spectrum that has such importance to polarization vision? Hawryshyn (pers. comm.) suggests that the underwater UV light field undergoes fewer changes during the day, and was more stable on an evolutionary scale, than other regions of the visible range. This stability is important when polarization sensitivity is used for navigation. Direct measurement of the polarized light field, at different parts of the spectrum, are required for better understanding of the information available from this type of sensitivity.

The question of the role of UV sensitivity in deep sea organisms, living in regions deficient in UV light, is anything but answered. What characteristics of the light field trigger vertical migration (Frank & Widder 1994)? Does UV sensitivity serve as a depth gauge for vertical migrating animals (Wald & Rayport, 1977)? Does the bioluminescent spectrum of some animals extend to the UV-violet region, to enable discrimination between animals (Frank & Case, 1988)? Detailed measurements along with behavioral studies are required to answer this enigma.

Goldsmith (1990) cautioned that it is anthropomorphic and naive to ask, "What does a UV receptor do" for animals? Nonetheless, pursuing answers for specific questions, such as those raised in this discussion, will enable better understanding of the role UV vision plays in the life of marine animals.

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Polarization vision as a mechanism for detection of transparent objects

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ABSTRACT: Polarization vision may be used by aquatic organisms to improve detection of targets. In this study we demonstrate that polarization vision can improve both contrast recognition and detection range. Target detection range increased by up to 82% for transparent targets, which depolarize light, while only by 12 - 14% for targets with an intensity contrast. A similar improvement in detection range is likely to exist for transparent organisms such as zooplankton, whose tissues depolarize light. Behavioral studies are needed to assert that polarization vision is used by planktivorous organisms to enhance detection of transparent plankton in the water column.

INTRODUCTION

Various marine animals, such as crustaceans (Ritz, 1991; Sabra & Glantz, 1985), cephalopods (Moody & Parris, 1960) and fish (Cameron & Pugh, 1993; Cameron & Easter, 1993) are sensitive to the orientation of the *e*-vector of light, or have some form of polarization vision. Although the record of such polarized light-sensitive animals is increasing, the function of this form of vision is as yet largely unknown. As in bees (Rossel, 1993), aid in navigation has been suggested to be an important role of polarization sensitivity and indeed grass shrimps use it to determine the direction of a shelter (Ritz, 1991). Octopus are able to distinguish between targets based on polarization vision (Moody & Parris, 1960). Contrast enhancement and detection range increase have long been suggested as possible roles for polarization vision. Detection range for targets can be increased by about 20% by using various polarization techniques (Briggs & Hatcett, 1965; Lythgoe & Hemmings, 1967). It is conceivable that detection of transparent targets will be enhanced to an even greater extent.

To some extent, the underwater light field is linearly polarized. Partially linearly polarized light can be regarded as a combination of two states; one totally depolarized with the intensity I_d, and the other fully linearly polarized with the intensity I_p (Kliger et al., 1990). The orientation or angle of polarization is defined as the orientation of the *e*-vector of the linearly polarized component, from the point of view of an observer looking at the source of light or reflection (Kliger et al., 1990). The intensity of the two states, when measured using an analyzer oriented perpendicular to the orientation of the *e*-vector of the linearly polarized state, is equal to $I_d/2$. When examined at the orientation of the *e*-vector of the polarized state, the intensity is equal to the sum of the two states ($I_d/2 + I_p$) . The total intensity is, therefore, $I_t = I_d + I_p$. The partial polarization is defined as I_p/I_t, and it ranges from 0 to 1 inclusive, where 0 indicates unpolarized, and 1 fully-polarized light (Wolff, 1990).

The underwater light field, especially in shallow water (down to 50 m) shows strong linear polarization in the horizontal plane (Tyler, 1963). This strong polarization presents a distinct background for any object which reflects or transmits light that is polarized at a different orientation or that is depolarized. Dinoflagellates are known to induce circular polarization of light passing through them, and numerous other species depolarize the light as it passes through their body (Fig. I). Many plankton species are largely transparent, and are therefore hard to detect by visual predators. It is conceivable that polarization vision will enable such predators to improve detection of their transparent prey. This will be especially true when the background water is polarized at a given orientation while the plankton is either polarized at another angle, has smaller partial polarization, or is completely depolarized. However, it has not yet been shown that, indeed, polarization vision can be used in this fashion.

In the current experiment we checked the hypothesis that polarization vision can improve detection of transparent, yet depolarized, targets.

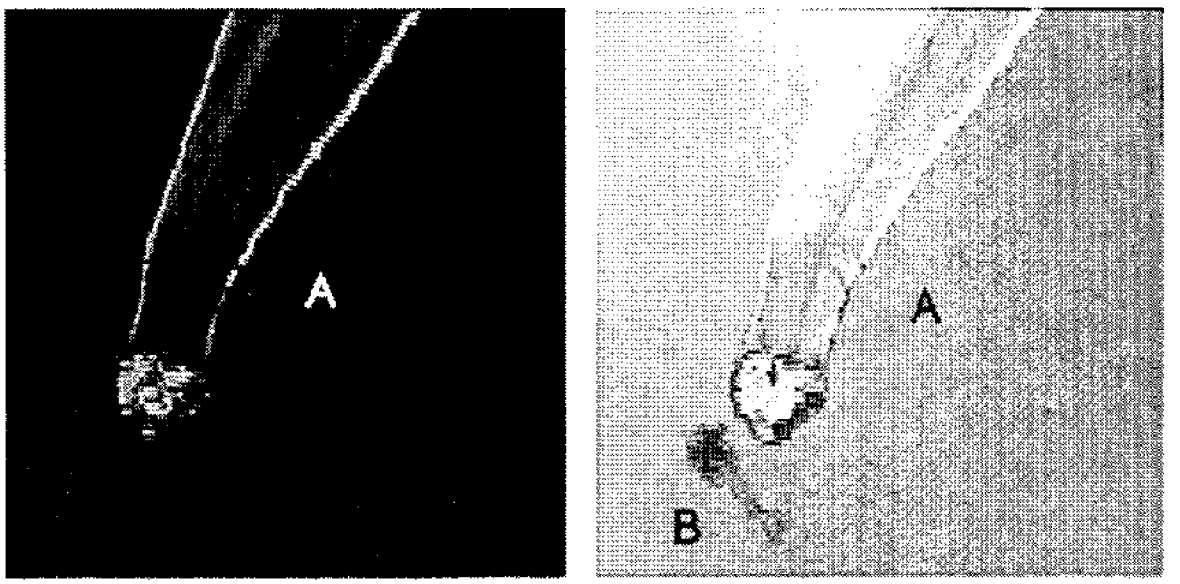

Figure 1. A chaetognath (A) and a zoea larva (B) viewed against "regular" back illumination (right), and through two polarizing filters at perpendicular orientation to one an other, one placed underneath and one above the sample (left). The light polarized by one polarizing filter is extinguished by the other. However, the tissues of the chaetognath act as depolarizers, and therefore can be clearly seen. The zoea does not affect the polarization orientation and is, therefore, not seen. Under "regular" illumination, both transparent animals are not easily detected.

MATERIALS AND METHODS

This research was conducted in a small mangrove channel on Coconut Island (Moku o Lo'e), Kane'ohe Bay, O'ahu; at a water depth of 2.5 m. Six targets were viewed through a polarization analyzing camera. For each of these targets the characteristics of the light were analyzed and the maximal detection range based on black and white contrast and based on polarization contrast were determined.

Targets were 16.5 x 16.5 cm, and made of transparent Plexiglas that does not affect the polarization of the light. Each target was divided into 1.8 cm wide stripes which were then alternatively covered by black or white totally-opaque tape, or a thin transparent plastic film that acts as a depolarizer. Coating was such that the targets had alternating stripes of either black and white, black and transparent (no coating), black and depolarizing transparent, white and transparent (no coating), white and depolarizing transparent, or depolarizing transparent alternating with no coating.

The polarization sensor used is based on the design of Wolff and Mancini (1992) adapted for portability and field work and will be fully-described elsewhere. To summarize, the sensor consists of two twisted nematic liquid crystals (TNLCs) of 45° and 90° rotation, and a linear polarizer (Polaroid® HN38S, which has a high and constant extinction coefficient ratio throughout the visible range) both placed in front of a small digital camera (Electrim EDC-1000 monochromatic digital camera with 192 x 165 CCD array) connected to a portable personal computer (PC). The TNLCs rotate light by 0°, 45° or 90°, by applying an AC current at desired times (controlled by the PC) and three images are acquired. These images are used for on-line analyses and display of the polarization characteristics of each pixel in the image. Our analyses program presents the intensity image ("black and white"), the partial polarization, and the orientation of polarization, on a single element resolution, throughout the image.

Targets and camera were placed horizontally at a water depth of 2 m (0.5 m above the bottom) where the camera line of view was perpendicular to the direction of the sun. All measurements were taken between 10:00 and 14:00 hours, when the inclination of the sun was relatively high and under cloudy sky providing a relatively constant light field.

Each target was viewed at 1 m distance from the camera, and the image characteristics (intensity, partial polarization, and orientation of polarization) were recorded from this setting. Targets were then placed at increasing distances from the camera, and the distance at which they

disappeared, depending on mode of vision, was recorded. For each target two modes were used; intensity (black and white) only and polarization (including intensity). In each case, the observer of the output display was aware of the mode used, but not of which target was used nor at which range it was positioned, until after final decision of detection range was made.

RESULTS

The natural light field in the water of this mangrove lagoon was found to be highly polarized. The orientation of polarization was horizontal (parallel to the water surface) and the partial polarization was 0.35.

Measurements of the characteristics of the light coming from the different targets, placed at a distance of 1 m from the camera, (Table I), showed a profound difference between the different types of coating, especially between the transparent and the depolarizing settings. Even at the short distance of 1 m, a considerable amount of polarization evolved from the scattering of light by the water. This short-distance polarization amounted to partial polarization of 0.07 to 0.33, close to the background polarization of the water. However, one must consider the possibility that a small degree of linear polarization was introduced by the reflection of light off the targets themselves. This may be the explanation for the difference in the average orientation of polarization of some of the targets from the background orientation of polarization.

Target detection range based on intensity contrast was substantially different from that based on polarization contrast for each of the targets (Table II).

In targets which contained a strong internal intensity contrast, polarization analyses increased detection range by 12 to 14%. However, when the fully transparent target (no coating and a depolarizer) was examined, polarization contrast analyses increased detection range by 82%.

Table I. Characteristics of the light (average ± standard deviation) measured for targets placed at 2 m depth, at a distance of 1 m. Targets were transparent Plexiglas (not affecting the polarization of the light), 16.5 x 16.5 cm, divided into 1.8 cm wide stripes. The stripes were then alternatively covered with black or white totally-opaque tape or with a thin transparent plastic film that acts as a depolarizer. This coating created a pattern of either black and white, black and transparent (no coating), black and depolarizing transparent, white and transparent (no coating), white and depolarizing transparent, or transparent depolarizing alternating with no coating. Intensity is measured on a 0 - 255 digitized scale.

Target type	Intensity (0 - 255)	Orientation of polarization (0 - 180°)	Partial polarization (0 - 1.0)
Black and white			
Black stripe	8.5 ±2.8	N/A	0
White stripe	66.31 ± 7.7	168 ± 7	0.13 ± 0.02
Ratio B/W	0.13		
Black and transparent			
Black stripe	13.80 ±3.1	N/A	0
Trans. stripe	42.00 ±8.0	172 ±3	0.34 ±0.06
Ratio B/T	0.33		
Black and depolarizing			
Black stripe	14.25 ±2.3	N/A	0
Depol. stripe	53.67 ±5.7	174±4	0.23±0.04
Ratio B/D	0.27		

Table I (cont.)..

Target type	Intensity (0 - 255)	Orientation of polarization (0 - 180°)	Partial polarization (0 - 1.0)
White and transparent			
White stripe	88.31±5.3	174±10	0.07±0.01
Trans. stripe	71.64±2.9	1±10	0.14±0.02
Ratio W/T	1.23	0.52	
White and depolarizing			
White stripe	57.20±4.2	3±4	0.32±0.06
Depol. stripe	37.27±2.2	167±5	0.33±0.04
Ratio W/D	1.53		0.94
Transparent and depolarizing	51.91±2.0	175±2	0.32±0.01
Trans. stripe	65.33±2.5	175±3	0.22±0.03
Depol. stripe	0.79		1.44
Ratio T/D			

Table II. Target detection range based on intensity contrast and on polarization contrast. Targets were viewed through a polarization analyses camera at a depth of 2 m. The natural light field at the horizontal direction, which served as background to the targets, was 35% polarization at a horizontal orientation.

Target type	Detection range (m)		Ratio
	Intensity contrast	Polarization contrast	Polarization/ intensity)
Black and white	3.5	4.0	1.14
Black and transparent	3.0	3.5	1.17
Black and depolarizing	3.0	3.5	1.17
White and transparent	3.0	3.5	1.17
White and depolarizing	2.5	2.8	1.12
Transparent and depolarizing	1.65	3.0	1.82

DISCUSSION

The partial linear polarization of the light field in sea water sets the stage for a polarization vision-based object's detection and possible recognition. This polarization is linearly polarized in the horizontal plane down to depths of over 50 m when viewed at the horizontal (parallel to the surface) orientation. However, the orientation of polarization is expected to change significantly when observed from other directions. Major changes can be expected to occur at the edge of Snell's window, where light which is refracted as it enters the water from the air, is in close proximity to light which is internally reflected from the water surface. Regions of high partial polarization, or of changes in the orientation of polarization of the ambient light, such as the edge of Snell's window, are expected to be the preferred background for polarization-based target detection.

Lythgoe and Hemming (1967) reported an increase of 20% in target detection range when viewed through cross polarizing filters. Our results sustain this observation for targets which contain internal contrast. For transparent targets which depolarize the light, the increase in detection range was much more significant, up to 82%. Such an improvement in detection range is likely to be of great significance for both predatory and prey organisms.

Polarization vision is known to be used for various tasks such as navigation, spatial orientation, and target recognition. Our measurements demonstrate that polarization vision could be used to improve detection of transparent targets. Several planktivorous animals are known to possess polarization vision. We propose that this sensing ability is used for better detection of transparent plankton in the water column. In the current paper we demonstrated the existence for such improved detection; we hope that following studies will show the actual use of it, through behavioral studies.

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Designs for submersible imaging polarimeters

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ABSTRACT: Numerous terrestrial and marine organisms are sensitive to partially linearly polarized light (PLPL). The natural light field in a large portion of the water column is partially linearly polarized, and many objects in water reflect light that is polarized at specific orientations. Although humans use polarized light, our inability to see it limits our study and understanding of its distribution in nature and of the information it carries. By placing two twisted nematic liquid crystals and a fixed polarizing filter in series in front of a CID camera,we have constructed portable polarimeters that analyzes the characteristics of PLPL in a full image, on a single pixel basis. The analyzed image can be presented as a false color image where hue represents orientation of polarization, and saturation represents the partial polarization. We introduce here two configurations of the polarimeter: an autonomous sensor that uses a camcorder for recording images that are analyzed at a later stage; and an on-line sensor, that uses a digital camera attached to a personal computer which controls and analyses the information. The current polarimeters are limited to the visible region of the spectra. However, their basic design is applicable to a sensor operating in the UV region as well.

INTRODUCTION

Like wavelength and intensity, polarization is an intrinsic property of every light beam. Although humans make use of the polarization features, we are unable to sense them directly. However, numerous animals are sensitive to the orientation of linearly polarized light. These range from invertebrates, such as crustaceans (Ritz, 1991; Sabra & Glantz, 1985), insects (Phillipsborn & Labhart, 1990; Rossel, 1993; Wehner, 1976) and cephalopods (Moody & Parris, 1960) to vertebrates such as fish (Cameron & Pugh, 1991; Hawryshyn, 1992; Cameron & Easter, 1993), amphibians (Auburn & Tylor, 1979) and possibly also birds (Phillips & Waldvogel, 1988; Phillips & Moore, 1992). This polarization sensitivity is used for navigation (Rossel, 1993; Wehner, 1976), spatial orientation (Ritz, 1991; Hawryshyn, 1992), and for detecting of large bodies of water (Schwind, 1991).

The underwater light field is strongly polarized down to considerable depth (Tyler, 1963). Such strong polarization could be used by a polarization based detection or identification visual system. This background polarization arises mainly from the scattering of downwelling light. We expect the UV portion of the spectrum, which is highly scattered, to be considerably polarized.

Although we are well aware of the visual systems of animals sensitive to polarized light, our current understanding of the information it carries is very limited (Hawryshyn, 1992). One reason for this lack of knowledge is our inability to visualize the different components of polarized light, namely orientation, partial polarization (named also "percent polarization" or the "amount of polarization" (Wolff, 1990)), and phase delay or circularity. The current discussion is limited to partially linearly polarized light (PLPL), and therefore the last component (circularity) will not be considered.

Partially linearly polarized light can be described as a mixture of two states, one completely depolarized, with an intensity of I_d, and the other fully linearly polarized having an intensity of I_p (Kliger *et al.*, 1990). The angle of polarization (or the orientation of polarization) is therefore the orientation of the *e*-vector of the polarized state. The intensity of the two, when examined through an analyzer oriented perpendicular to the orientation of polarization, equals $I_d/2$, and when examined at the orientation of polarization it equals ($I_d/2+I_p$). Therefore, the total intensity (I_t) equals I_d+I_p. The partial polarization is defined as I_p/I_t, and ranges from 0 to 1, where 0 indicates unpolarized, and 1 fully polarized light.

A method of presenting PLPL which can be readily understood was developed by Wolff (1990). The angle of polarization is multiplied by 2 (to achieve a 360° range) and according to this value, it is assigned a false color (hue) on the 360° HVS (hue, value, and saturation) scale. The partial polarization is assigned to saturation on a 0-1 scale, and the total intensity (I_t) is assigned to value. Therefore, depolarized light will appear on a gray scale, while completely polarized light possesses a saturated hue which depends on the orientation of polarization.

MEASUREMENT TECHNIQUES

Measuring the characteristics of partially linearly polarized light involves quantifying three parameters: intensity, angle of polarization and polarization. Naturally, this involves three separate measurements. For instance, imaging devices such as film or video cameras could be used with linearly polarizing filters positioned at three separate orientations in front of them. Designs suggested include using two or three cameras, each equipped with its own filter (Lythgoe & Hemming, 1967; Bernard & Wehner, 1977), or rotating a filter in front of a single camera (Briggs & Hatcett, 1965). Each of these approaches has particular advantages and disadvantages. Using three separate cameras permits simultaneous acquisition of all three images, but has inherent magnification and parallax limitations. Additionally, precise corrections are required to compensate for small variations between cameras. Rotating a filter in front of a single camera solves the parallax problem but introduces potential distortion due to surface inconsistencies and to alignment limitations of the filter. Further, since the three measurements are taken in sequence, there is a limitation in the ability to measure changing events. The introduction of twisted nematic liquid crystals (TNLCs) (Wolff & Mancini, 1992) solved the inconsistencies caused by the filter rotation, as the same effect as filter rotation is achieved without the use of any moving parts. However, the time limitation, resulting from the fact that three measurements are taken one after the other, is still an intrinsic limitation of this type of sensor.

Twisted nematic liquid crystals (TNLCs) have a helical molecular structure, twisting gradually from one face of the crystal to the other, by a number of degrees (n) that can be controlled during manufacture. When a voltage is applied across the TNLC, the molecules are stretched and the twist is straightened. When the voltage is 0, the molecules return to their twisted stage. The orientation of polarization of light applied across the TNLC follows the molecular structure and rotates by n degrees when the TNLC is relaxed, or does not change when it is stretched. Some of the advantages of TNLCs are that they do not change the geometrical arrangements of the incoming image, and that they transmit light across a wide-spectrum. They are limited, however, in the time required for change in position (stretch or relaxation). In the device described here we used fast TNLCs with a switching time of 16 ms. By applying two TNLCs in series in front of a linear polarizing filter, one can obtain the same information as if the polarizer was rotated to as many as four different positions, without having any mechanical motion. Only three orientations are needed for measurement of PLPL; we chose to use the 0°, 45° and 90° orientations.

THE POLARIZATION SENSORS

The devices described here are based on the design of Wolff and Mancini (1992) adapted for portability and field work underwater. Two models are described: a totally autonomous device, based on a camcorder, where data are collected in the field, but analysis is conducted as a separate stage, and an on -ine digital sensor connected by cable to a personal computer.

The autonomous sensor consists of two TNLCs of 45° and 90° rotation, and a linear polarizer (Polaroid ® HN38S, capable of polarizing light at wavelengths from 400 to 700 nm, with a high and constant extinction coefficient across this range) both placed in front of a Yashica KX-V1 camcorder (Fig. 1). Images are taped on Hi-8 video format, and the state of the TNLCs is independently recorded by placing small polarizing filters in the field of view of the camera, attached to the sensor's underwater housing. Sequences of individual fields of images from the three desired orientations, are transferred, as 320 (H) X 240 (V) pixels, 24-bit, color images, through a VideoSpigot™ frame grabber board, to a personal computer for analysis. Each pixel in the frame is analyzed separately. This sensor has the potential to acquire fully-analyzed images at one third of the video rate or 10 Hz, but in most field work it is used at 5 Hz or slower, to insure that at least one full frame is available at each polarization position. Once transferred to the computer, each color channel can be analyzed separately, providing an estimation of changes in polarization between different parts of the spectrum, or they can be recombined to create an 8-bit black and white image, and provide information on the over all light field.

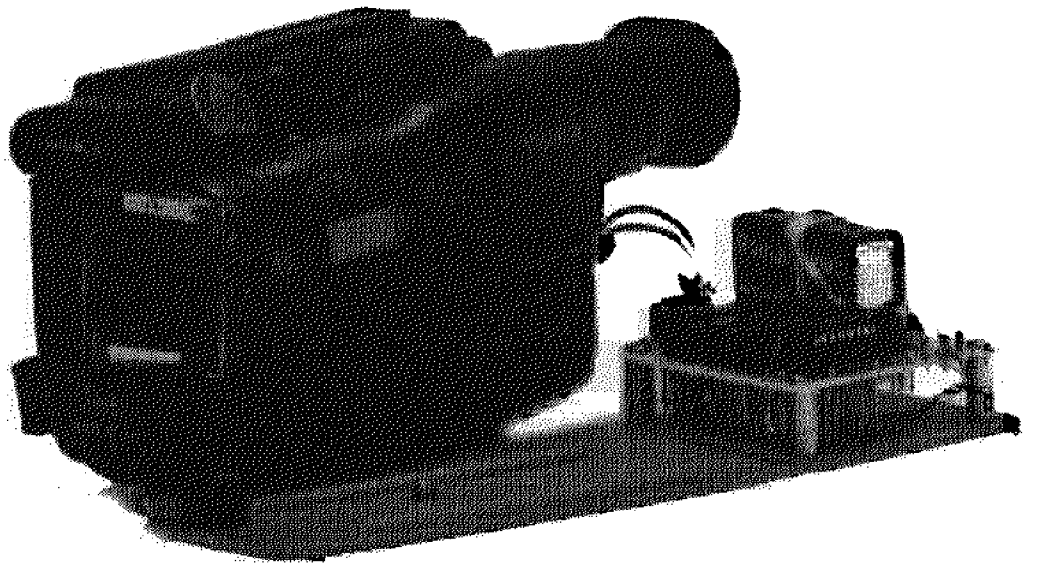

Figure 1. A video camera based polarization sensor. Two TNLCs and a Polaroid HN38S polarizing filter are mounted through a standard 37 mm ring in front of the camcorder lens. The controlling circuit and a 9 V battery, used to power and stretch the TNLCs, are placed on the same slate used to hold the camcorder in the underwater housing. This sensor can be fully autonomous and does not limit the operator's movements. Images are transferred to a computer for analyses at a later stage.

The on-line sensor uses a similar sequence of TNLCs and a polarizing filter, but in this case they are mounted in front of a digital camera (Fig. 2). We use a monochromatic digital camera (Electrim EDC-1000) equipped with a small CCD array (192 pixels horizontally x 165 pixels vertically) to enable fast transfer of the image to the computer. In the trade-off between resolution and transfer rate, one may choose larger CCD arrays as well as faster cameras, according to the desired application. The images are transferred by cable to a personal computer ("lunch box" type made by Bi-Link Computers, with a 486DX2 board), analyzed, and presented as a false color image. This system is temporally limited by the capture and transfer rate of the camera as well as the time required for analysis, but the results are available immediately.

Both sensors can be placed in underwater housings for submerged field work. The video camera-based sensor is fully autonomous and can be carried by a single diver. The sensor based on a digital camera requires connection to the PC and good communication between the person controlling the camera and the one running the software and viewing the images.

ADJUSTMENTS FOR THE UV RANGE

A growing portion of the literature shows that vision in the ultraviolet (UV) range (300 - 400 nm) is closely related to sensitivity to PLPL (Wehner, 1976; Hawryshyn, 1992). Further, the characteristics of PLPL are expected to change according to the wavelength observed, and should be especially strong in the short end of the spectrum. Therefore, we have a great interest in designing a polarization sensor that will operate in this range. Adjusting the current sensors for work in the UV range means, in fact, constructing a new sensor dedicated specifically to this task. The changes required are in several components of the sensor; the TNLCs, the sensing device, and the housing of the sensor. The TNLCs should be adjusted for maximal rotation of the light at about 350 nm, and should be put between quartz plates (currently, they are set between glass plates) that will not absorb the light. The polarizing filter used with the TNLCs should have a high extinction coefficient in the UV range, such as Polaroid's HNP'B filter. The sensing device itself should be designed specifically for working in the UV range (300 - 400 nm). This will require using a UV-sensitive camera, such as a charge injected device (CID) based camera, with lenses that

transmit in the UV range, and proper filters that will block the longer end of the spectrum. The housing of the camera should contain a UV transparent window through which the light can penetrate to the sensor. However, the analysis and control software currently used can be useable for the UV sensor. Though the adjustments are substantial, the information expected to be gathered is well worth the effort.

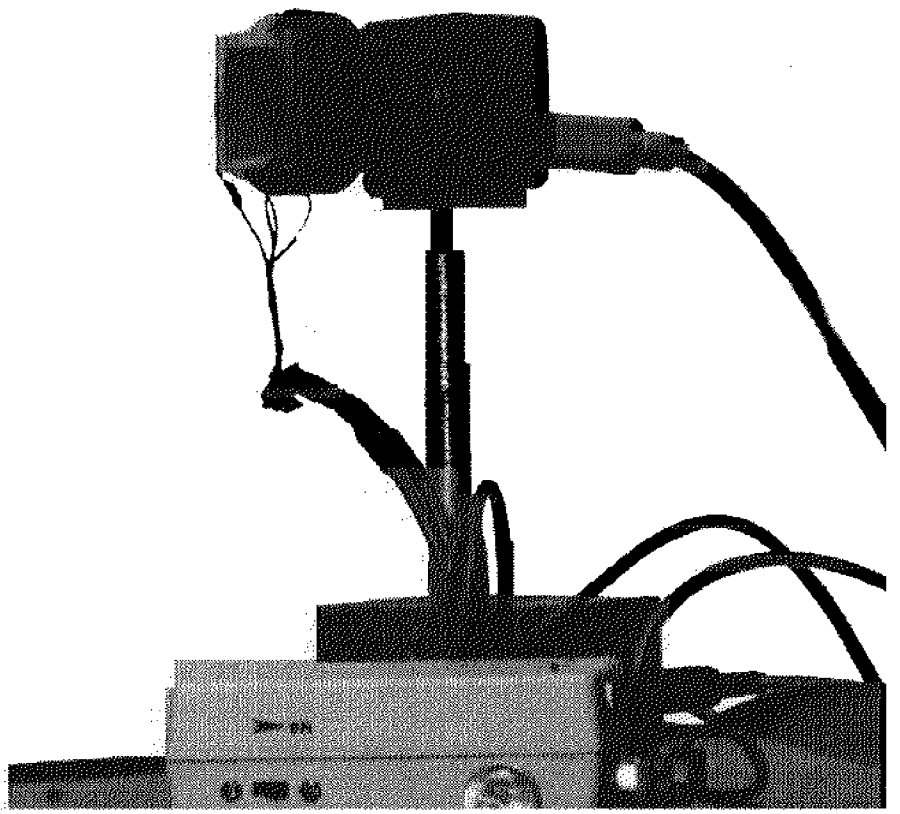

Figure 2. A digital camera-based, on-line, polarization sensor. The two TNLCs and the polarizing filter are mounted in front of an Electrim EDC-1000 monochromatic digital camera. The positioning and switching of the TNLCs are controlled by a "lunch box" type, 486DX2, personal computer through a separate circuit. Eight-bit images, 192 (H) X 165 (V) pixels, are transferred to the computer where the polarization characteristics are analyzed and displayed

FURTHER RESEARCH AND DEVELOPMENT

Numerous animals are sensitive to linearly polarized light and use it for various tasks. Due to our inability to visualize the distribution of PLPL, our understanding of these tasks is very limited. Our imaging polarimeters are already being used in the field to measure the natural polarized light field, and the light reflected from objects. We expect these measurements to provide an insight to animal polarization vision. As an additional step, we hope to adapt the sensors to work in the UV (300 - 400 nm) range of the spectrum.

The present paper describes a design of a field-operational, submersible, imaging polarimeter. We believe that our design can be used in other field-oriented studies. We expect this method of studying and presenting PLPL to open a window to aspects of the visual world, currently obscured from our eyes.

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Ultraviolet imagery

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The recent discovery of possibly wide-spread ultraviolet-sensitive vision in marine animals casts a new light on the need for research on ultraviolet radiation. Weiler (1993) outlines the results of ultraviolet radiation workshops dating back to 1985. Throughout her summary, the central biological theme is *Defense* against damage caused by UV-A and UV-B radiation. In the current workshop, Dr. David Mauzerall cast a somewhat different historical view on the possible importance of UV radiation in the origin of life. The biological entities that today must defend against damage from UV radiation may owe their very existence to this same radiation as an energy source for the origin of complex organic molecules. Recognition of the potential importance of UV vision in marine animals indicates a present-day beneficial influence of UV radiation: various marine animals have been selected for visual sensitivity to the same UV radiation that has led to defensive mechanisms in other systems. This section of the workshop explores our current knowledge regarding the occurrence and function of UV vision, the physical parameters of UV radiation that must be explored and the engineering specifications for the equipment that is required to study these systems.

PRESENCE OF ULTRAVIOLET VISUAL SENSITIVITY IN CORAL REEF FISHES

Dr. William McFarland reviewed the biophysics of monochrome and color vision in animals and gave special attention to the marine environment. Signal detection theory is especially important in considering the degree to which the absorption spectra of visual pigments match the spectra that are available in the background of the visual field and in the reflection from the object to be detected. Given this background, the potential importance of UV vision is seen as far more than just an ability to detect the coloration of an object. We must consider the UV absorption patterns of objects in the marine environment along with the nature of the spectral distribution of light in the environment and the sensory sensitivity to all portions of the spectrum.

Over the last decade the presence of ultraviolet visual sensitivity in various vertebrates has been demonstrated by behavioral tests. The basis of UV sensitivity has been confirmed by spectrophotometric analysis, which has revealed a class of single-cone photoreceptor cells that contain visual pigment that absorb light maximally between 350 and 400 nm. UV vision in birds is used in navigation and, in hummingbirds for example, as a unique visual means of guiding a pollinator to a flower's nectar (nectar guides). In lizards UV vision is used in courtship displays (Fleishman, Loew & Leal, 1994). In some fresh water fishes UV vision enhances the contrast of zooplankton (Loew *et al.*, 1993), and in goldfish (Hawryshyn & McFarland, 1987) and salmonids (Hawryshyn, 1992) it provides information about polarization fields that is useful in orientation and navigation.

It was recently shown that some coral reef fishes also possess this unique visual capacity (McFarland & Loew, 1994). Damsel fishes, which are highly territorial, display distinct courtship rituals, and lay demersal eggs, have visual pigments that absorb near 360 nm. The behavioral function of these visual pigments, however, is unclear. Possibilities include foraging, mate recognition due to "unseen" UV markers, agonistic display signals, UV-polarization detection for contrast enhancement and, perhaps, for orientation and navigational movements within the reef community. It is likely that this unusual visual ability is widespread among reef fishes, and especially so, because UV-A light penetrates in coral environments to considerable depths (30 or more meters) and at a high enough intensity to provide readable optical signals (McFarland, 1985; Loew & McFarland, 1990). It is important, therefore, to "visualize" how coral ecosystems appear in

near-UV light. This can be achieved most efficiently by the use of a UV-sensitive, video-based imaging system.

Dr. Craig Hawryshyn continued the review of the broad spectrum of evidence for UV vision in aquatic and terrestrial vertebrates (see *Vision Research* volume 34 #11, 1994 for special issue on UV reception in animals). Few studies have emphasized the characteristics and dynamic features of UV photoreception, but there is some limited information on the performance characteristics of UV cone photoreceptors in relation to the other cone photoreceptors of vertebrates. For instance, Hawryshyn (1992) examined the light adaptation of cone photoreceptors over a broad range of ambient intensity. From this data, we can extrapolate to the photic regimes under which these cone photoreceptors would normally operate and hence understand the conditions suitable for visual behaviors. Our limited knowledge suggests that UV cones have more in common with the rods than the other cone photoreceptors in terms of the intensities of ambient light under which the cones operate (dynamic range tuned to twilight conditions). The advantage of having such data is that it helps in answering questions related to how the receptors may be employed by the organism to gather information vital to guiding their behavior. Another such study by Hawryshyn and McFarland (1987) examined the response of fish to plane-polarized light. This study as well as others (see Hawryshyn, 1992 for review) have brought to light the complex nature of how vertebrates detect and process plane-polarized light. Various studies have shown that the cones respond differentially to the plane of polarization and that this may be used to enhance contrast of the images, but direct evidence to support this contention is not forthcoming. The role of UV-polarized sensitivity in orientation and navigation has been established in the laboratory, but open ocean studies are still in the planning stages.

Hawryshyn's group has identified two main research areas to pursue: (1) Optical signaling: We have literally no knowledge of intraspecific and interspecific modes of visual communication in the ultraviolet spectrum. For instance, current studies of optical signaling in the cleaner wrasse address signals that are displayed by the cleaner wrasse that evoke a posing behavior in the host species. In recent UV video photography during a post-workshop session (Dr. E.R. Loew), it was clear that juvenile and adult cleaner wrasses had marked differences in the pattern of UV reflectance. (2) UV polarization sensitivity may play a role in guiding orientation movements of fishes on and off the reef especially during dusk and dawn, a period of greatest polarization in the light field. Whole-field imaging of polarization patterns, or a "fish's view" of the polarization field in the UV and "visible" spectrum as planned for the UV imaging system, would enable a much deeper understanding of the possible importance of this capability.

SPECIFICATIONS FOR A VIDEO-BASED UNDERWATER IMAGING SYSTEM

What is there to see in the near-UV visual world of marine animals? Dr. Ellis Loew summarized the conclusions of the participants as to the type of equipment that is needed to answer this question. There are three visual mechanisms that must be considered. First is simple luminosity; that is, are UV images coded as gray-scale without the potential for using the UV information as part of a color vision scheme. Obviously, color vision is the second potential use for UV information. Here, one must consider whether there is enough differential reflection of UV for hue discrimination in this spectral region to be useful. Lastly, there is polarization processing of images in the UV. Any camera system to be designed should be capable of providing information in all three of these areas.

The Lens:

This is the most important part of any UV imaging system and also the one item that is not currently available 'off-the-shelf'. The ideal lens would be almost identical to the zoom systems on personal camcorders -- 30 to 80 mm motorized zoom, manual- and auto-iris, and macro ring. Unfortunately, to obtain such a lens with transparency into the near-UV is not really feasible and would be a research project in itself. Rather, a number of C-mount, quartz achromatic lenses of different focal length and corrected for the 300 to 400 nm region can be turret mounted with some kind of

remote switching mechanism. The 300 to 400 nm correction region for optimization of the lenses is specified for visual system research. However, if coatings are to be used they should be very broad-band so that images could still be obtained in the shorter UV regions for use in other research areas. The faster the lenses, the better, as the amount of UV light is not great given the sensitivity of most unintensified cameras. A lower limit is probably about f/4.

The easiest way to focus the system would be to adjust the detector-to-lens distance with a linear motor or stepper. Many cameras used for remote sensing already have this capability.

Trials with Dr. Loew's current underwater UV-sensitive camera demonstrated the need for a highly-corrected, multi-lens system. His present camera, specialized for visualization of zooplankton in the UV, uses an 'off-the-shelf' quartz meniscus lens. This camera is well-suited for its intended task, but could not provide images of the quality required for field study of reef animals or pelagic fishes.

The Filters:

Next to the lens, these are the most critical elements. The ideal filter is a rectangular function having 100% transmission over its designated bandpass and zero transmission out of band. Of course, such ideal filters do not exist and there are many tradeoffs that must be made in choosing a filter. For imaging, the best filters are absorptive, like the common Hoya U filters or the Kodak 18A. These have excellent optical clarity, low scatter, no pinholes and are spectrally independent of angle-of-incidence. Such is not the case for thin-film interference filters. Unfortunately, all absorptive UV filters have a red-window which leads to contamination of the UV image. For underwater work, this may not be that significant a problem as water filters out much of the red light in this window anyway. However, this problem must not be ignored since the camera could be used in surface or on-shore imaging.

The best design would use absorptive filters for band isolation with thin-film IR blockers such as those from Omega Optical. Narrow-band absorptive filters are hard to come by and for this kind of filter, thin-film edge filters along with the IR blocking element can be sandwiched with the UV isolator.

For luminosity imaging, the bandwidth should cover the 300 to 400 nm range. For chromaticity imaging, a series of filters of narrower bandwidth would be needed. Five filters with 20 nm half-band width would be ideal. These would be mounted in a filter wheel behind the lens turret and could be chosen via an indexing motor.

The polarization 'filter' is placed in front of the lens and should be detachable. Electrically activated polarization filters for use in the UV are currently being developed and specifications for this filter will be available from Dr. Tom Cronin.

The Camera:

This is really the easiest part as many commercial camera systems are out there. Obviously, one wants a robust camera with remote operation potential. After this comes the choice of detector. While tube-based cameras offer a number of advantages in terms of spectral sensitivity as the phosphors can be specified for the UV, a solid-state camera is preferred due to inherent ruggedness. The best camera at this time (CIDTEC of New York) uses a Charge Injection Device (CID) detector with high inherent UV sensitivity and a quartz window. Another advantage of the CIDTEC camera is the ability to control integration time of the camera for electronic shuttering while watching the integrated signal appear on the screen. This makes shutter timing easy to deduce. Various hand-held and remote controlled (pan & tilt) housings are commercially available or already on-hand at HIMB.

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DISCUSSION AT WORKING SESSIONS

Moderated by Dr. Zvy Dubinsky and Dr. Paul Jokiel

Editorial note: Extensive discussions of research needs occurred throughout the summer research program as well as during the workshop. The section below is summarized from the "round robin" working session at the workshop in which participants had the opportunity to state their major concerns and interests. This dialog summarizes much of the interaction that took place throughout the summer, and demonstrates the fertile sharing of widely diverse ideas. Many of these topics are being actively persued by the participants.

Dubinsky: If we want to progress, I think we should try to distill the knowledge. We need to compartmentalize the different areas of interest and different levels of resolution and understanding of the problem. Maybe we should try to divide questions into biophysical, physiological and ecosystem levels and what we would like to see evolving in terms of instrumentation and what research questions we would like to develop in each of these three fields.

Another thought is that we should look at controls. For example, everybody has been doing the same kind of experiment. PAR versus PAR plus UV. This is the classical experiment for everybody here. Is PAR alone the proper control for PAR plus UV? Maybe the control should be an equal number of total quanta. For example, if we subtract UV should we add more PAR so that the system gets the same amount of photons delivered. But it may be even more complicated! Maybe we want to see the same number of photons absorbed. So then we need to take into account differences in absorbance coefficients for the system for UV and the visible. Some of our action spectra are not action spectra in the same way they are defined in biophysical research. This was brought up in the discussion of time scales. But it is not just a question of time scales. For example, in Satoru's talk he mentioned the formation of a chemical induced by UV. But that chemical is also induced by high light. Maybe if we increased the PAR by that extra PAR equivalent to the dose of delivered UV, we would have ended up with the same results.

I think also that our definitions and our conceptual tools, at least in the same mechanistic compartment, are still very fuzzy. I would like to see a lot of development on this side. If we try to mix the tools that are needed to understand the basic mechanisms of absorption and initial action of UV, and then try to move to physiological and subsequent ecosystem levels and relevant questions, we must move in an orderly way. We must start from the foundations which are basically the molecular processes and definitions which have been worked out.

There is also a concern that we standardize our approaches and methodology, at least in the initial stages. We need to clearly define terms and concepts and experimental protocols, measurement, etc. Is there a need for a workshop on this?

Chadwick-Furman: We should look at the enhanced effects of UV on coral. There is a demonstrated need to look at long-term versus short-term effects, at least over a scale of several months, and to include long-term effects on coral growth, especially in terms of populations and communities, and the differential effects on different species.

Stambler: We should look at symbiosis, the relationship between zooxanthellae and animals, and how this relates to coral bleaching, etc.

Santos: We should solve the mystery behind MAAs. What is the purpose of MAAs? Are they really UV blockers? There has been no validation of this!

Taguchi: I would like to see somebody work on the vertical mixing of the phytoplankton. What is the optimum environment, the physical environment, in relation to the physiology of the cell?

Holhbauch: I think we should get more of an overall picture of an area, including UV measurements, analyze for MAAs, etc. Then, manipulate those corals by transplanting them to a new environment.

Response: (Patterson) We need to look at the integration of scales and the integration of resources when we look at remote sensing and monitoring. Satellites give us different scales; different time scales and different spatial scales. What varies on what scale? We need to tie everything together. How do you integrate satellite data with site specific studies? What kind of overall experimental scheme? We need to know what is appropriate for what is being studied and what is appropriate for certain situations. Different scales require different instruments. There is a problem with communication and integration. Is there a place for satellites and what can we learn from remote sensing about coral reefs?

Mauzerall: We should try to identify the receptors and processes for these UV-A and UV-B effects. Lets find out what is causing this!

Saito: I want to know how much fluctuation of UV radiation within the same day and at the same latitude might affect experiments. John Morrow showed us yesterday how UV radiation changed with ozone concentration. So how does this effect the optical components of an experiment? Biologists need to have more refined data on the variation and intensity of UV.

Suttle: There is an assumption that UV-B is having an effect, yet we have no way to measure dose. We need to be able to get a handle on dose. Dose equals the multiplication of incidence times response (the biological or physiological response). That is the axis on all our graphs. We need to quantify the action spectra on our graphs. Dosimetry, irradiance field, and biological responses all need clarification.

Fisher: We need better information on the impact of ultraviolet on the basic processes involved in photosynthesis e.g., a better understanding of the molecular biology of primary producers such as phytoplankton.

Yaskobi: The connections between vertical and horizontal water motion and phytoplankton are very basic and we need to know, on a medium temporal scale of a week or two, how they effect the community.

Reatka-Kudla: One of the most important problems we face is the application of experimental results to broader scales in the field to understand some of the mechanisms involved in experimental biology. How can we apply our experimental results on a scale large enough in the field to predict what will happen to those communities (e.g., on the scale of a reef) if environmental conditions change?

Gitleson: There is a misunderstanding that remote sensing is useful only from satellites and on a global scale. Coral systems are very complicated and it is necessary to clarify how to go about remote sensing and how we can best use it as a tool. We need to work on local scales and then make predictions. We need to first understand underwater light fields, diffusion, and the continuous coefficient for downwelling and upwelling irradiance. And we need to be able to measure sub-surface irradiance. Only then can we predict what should be determined from space or airborn systems, sensitivity, etc.

Morrow: I think that we need to look at UV-A and UV-B as terms. The generalized expression of the hypothesis is that specific spectral regions will impact the typical coral photophysiology differently. We need to identify where the relevant spectral regions are and try to focus on them independent of buzzwords.

Crosby: It is extremely important to have a cheap, simple way to measure UV intensity and its integral, especially in tropical locations where instrumentation may not be available. Whether it is a chemical actinometer or a physical measure doesn't matter.

I also think there should be a central location for tropical ultraviolet research, someplace with coral reefs, that is readily accessible, and already equipped to directly measure the action of enhanced UV on a coral community.

Jokiel: The critical factor has to do with the energetic cost and material cost and limitation of adaptation of each of these cases. What is the level of cost at the organism level?

Blanck: It would be interesting to determine what the pathways or mechanisms of action of UV on reef organisms are, particularly the immature stages of corals, so that we can determine their levels of sensitivity.

Amrami: I would like to get an overall picture of the energy budget of, for instance, coral and what the influences are of UV-B on this system. What does UV actually do to the coral? I am also interested in the synergistic effect of temperature and UV. With the concern for global warming we should put both together. What can we do? What should we do? Scientists have a responsibility to mitigate and educate.

Shashar: I would like to measure UV properly, in crevices etc., the microhabitat. Is it really affecting the coral? There is a need for instruments that are portable and easy to carry around, that measure full spectrum, that are fast, that connect to fiber optics (2 pi and 4 pi), and that can measure through an angle or a full 2 pi. And to do it at the coral, at the interface, to develop a better sense of UV measurement. It could either enhance UV by a prism or allow you to block out specific wavelengths.

Bidwell: I would like to see a better understanding of the evolution of metabolic pathways, especially MAA pathways and genetic repair mechanism pathways, in order to better predict the effects of increased UV radiation on marine organisms.

Peachey: We should know the community level effects of UV with a more detailed community analysis that would go along with whatever experiment we decide to do in the field.

Kuffner: If you could find a primer of a gene involved in the biochemical pathway of the MAAs, then you could attach a labeled probe and dose natural samples to find out what tissues are producing the MAAs.

Baker: I think that we have underestimated the planktonic dispersal phases of benthic organisms, and UV could be a significant factor in these and other stages of the life cycle. I think we need to do experiments that boost UV rather than eliminate it, and to think more about our controls.

Banaszak: I think we should work on action spectra, especially in terms of time costs of action spectra, and relate that to dose and dose rates.

Ondrusek: I am also interested in action spectra. I think there is a problem with people using narrow bands or different filters (other than mono- or polychromatic methods) that cut off everything shorter than that wavelength. We need to use narrower bands of wavelengths that are enhanced by prisms or that are used to block out specific wavelengths. By using UV blockers, chemical filters (in liquid solutions), it should be possible to filter out narrow wavelengths. We need to figure out action spectra either enhanced by prisms, or if you want to block out specific wavelengths, keep everything and say you are cutting off or omitting things that might react.

Butow: I think we should be looking at biochemical selection, "survival of the fittest," on a biochemical scale, including repair mechanisms, and we need to look at the effects of UV on the microbial ecology of coral reefs. For example, lots of work has been done on the effects of UV on bacteria and viruses. There must be a nutritional aspect to that somewhere.

We have been removing UV in our experiments and I think enhancement is what we should be doing in the future. Why not do both in the same system? Also, we should look at both the negative results and positive results and integrate all of that into an understanding of what is going on.

Lewis: As a biologist, I would like to have better information on the UV field and potential increases and time scales involved. We should be looking at the effects of enhancement of UV, especially long-term, on coral growth. What is ecologically relevant or most likely to occur in terms of what these organisms are likely to see? What are the realistic levels of increased radiation that we might see?

Response: (Patterson) As far as ozone, there is annual cycle of increase of ozone of about 280-310 Dobson units. There is an 11-year cycle of the output of UV radiation and a 2.5-year cycle of ozone with many different scales and it depends on how they get put on top of one another. This is for the tropics. They say that with the ozone depletion over the top of these cycles they are expecting a 2 - 3% difference over the next couple of decades. The satellite imagery has been done polared at 30 - 60 degrees and over the marine areas the satellite has a problem of clouds.

Grotelli-Everett: No one has addressed the potential of a positive impact of any increase of UV might have. How, for example, under different environmental conditions, viruses in algae could potentially have the host infect the alga and get rid of them during a bleaching event? There is a potential for there to be positive effects. Also, not all corals are effected by bleaching or enhanced UV and there is a lot to be learned from them. There are a lot of answers to our questions that are to be found by using those organisms.

Gulko: We should have workshops on other ecosystems. We should look at other systems including mangroves, seagrasses, estuaries and freshwater streams; places where you have recruitment and that are important for lots of animals. But no one is looking at them!

Also, we have people who use different methods. It would be helpful if we could come up with a chart comparing radiometers, chemical and viral actinometers, and include costs, nanometer ranges, their pluses and minuses, accuracy, weights, etc. that are available for experimental design, including their manufacturers, by the people who actually have used this equipment.

Krupp: I am interested in understanding the connection between biochemical effects and the organism's response and what adaptive significance, if any, may be attributed to a particular organism's response. For example, I am interested in Paul's observation of planulae release when corals are exposed to normal UV as opposed to when UV has been removed. And that there is a reduction in growth under UV compared to no UV. Has there been a biochemical shift in energy budget? Is this an adaptive response on the part of the coral? What is the underlying biochemical mechanism?

Lowe: I like the idea of Gulko's that we should be looking at aspects of the tropical marine ecosystem other than zooplankton or phytoplankton and include freshwater systems.

I would like to look at UV as a selective pressure that animals must adapt to and how, over the long run, animals are going to respond to either increases or decreases in UV. Are there things that are akin to heat shock proteins? This cannot be the first time that organisms on earth have faced changes in the UV environment. We know that animals moving to new

environments are going to experience tanning responses. We are acquainted with what terrestrial plants do with increases in UV. Is there something akin to tanning in chlorophyll-containing marine organisms?

McFarland: Calibration is a very complex topic and not easy! I would like to recommend that there could be a few standards or suggestions that all of us could follow within the limits of our ability. I think we could compare our data bases a lot more readily. A couple of sources or calibration instruments or different sources would be worthwhile. Some standard guidelines would be worthwhile. UV-A works in radiance and vision, but UV-B may be important in lethal and sub-lethal effects.

Is there research, directed research, on developing new kinds of actinometers? There is some work being done with caged compounds, compounds that can be tuned to 340 nanometers and used for intracellular measurements of things. With a biosensor and an electrical readout it is essentially an actinometer and can be tuned very specifically, for example, the 340 line. Other possibilities may exist for these compounds.

Hawryshyn: I think we need to address some of the more subtle effects of UV that are important, if not obvious, on the surface. For example, you may be interested in photoreceptors and the degeneration of photoreceptors in fishes because of a high photon capture. This can, over time, lead to a disruption in the interactions between animals. Coral reef systems provide optimum environments for looking at optical signaling. If color vision systems of coral reef fishes are impaired, there could be reasonably profound implications on the biodiversity of coral reefs including fishes. What strategies should be used to look at these questions?

Cronin: I think it is important to have a better understanding of the light field within the specific organisms being examined. The discussion on action spectra is very important and the action spectra will change with the induction of whatever protective pigments or whatever response there is. There is not an action for everything in general.

I don't think there is even an action spectrum for a particular organism unless you specify what level of induction there is. So, understanding the processes that an organism goes through to protect itself from UV is critically important. Part of that is understanding what the pigments are that it will protect itself with and how the light field within the coral exists. And that includes penetration from the top, or perhaps the sides, and how much light there is. Chemical probes are particularly useful. Based on their polarity, you know where they are going to be. It would be helpful to develop lipid-based, semi-solid chemical probes.

Cox: The emphasis should be put back on the tropics as being areas that have many diverse types of UV environments, characterize those environments, and look at how organisms have adapted to the environments.

Te: Coral larvae settle in cracks and crevices. How do larvae find a place to settle? There are compounding factors that affect the environment including how UV impinges on the organism. It is important to know how all of these complicating factors such as salinity, rainfall, and suspended and particulate matter impact the organisms along with UV.

Open Discussion & End of Session.

Ultraviolet Radiation and Coral Reefs. 1995.
D. Gulko & P. L. Jokiel (eds.). HIMB Tech. Report #41. UNIHI-Sea Grant-CR-95-03.

APPENDIX I:
1994 Edwin W. Pauley Summer Program in Marine Biology
UV RADIATION ON CORAL REEFS
Course Syllabus

Zoology 715, Topics in Invertebrate Zoology, 4 credit hours
Instructors: Dr. Paul L. Jokiel (HIMB),
Prof. Robert A. Kinzie III (UHM Zoology), Dr. Michael Lesser (UNH Zoology), Prof. Donald Crosby (UC Davis, Dept. Environmental Toxicology), Dr. David Krupp (WCC).

Date	***Day***	***Time***	***Lecture***
7/5	Tue	0815	**Jokiel:** Intro I - General Introduction to Physical, Chemical and Biological Properties of Solar Radiation with Emphasis on UV. Intro II - Historical Overview of UV on Coral Reefs
7/6	Wed	0815	**Crosby:** Intro - Marine Photochemistry, Anthropogenic Changes in the Ozone Layer and Possible Consequeces
7/7	Th	0815	**Gulko/Jokiel/Lesser**: UV Reaching the Surface of the Earth, With Emphasis on Kane`ohe Bay. Temporal, Seasonal Variation, Relationship to Other Factors. UV Optical Charecteristics of Natural Water, With Emphasis on Kane`ohe Bay
7/7	Th	1300	**Lesser**: Demo. and Discussion of Use of Spectroradiometers on Reefs
7/8	Fri	0815	**Discussion SEMINAR (Jokiel):** 1)What is the Overall Impact of UV on Coral Reefs? 2)What is the Potential Effect of Predicted UV Increase? 3)Do We Know Enough to Say Anything About the Possible Dangers?
7/11	Mon	0815	**Crosby:** UV Photochemistry - Actinometers, Dosiometers
7/12	Tue	0815	**Kinzie**: UV and Coral Metabolism
7/13	Wed	0815	**Crosby:** UV Photochemistry - Phototoxicity and Photodegredation
7/14	Th	0815	**Lesser:** Action Spectra -What is it and how do we apply it?

7/15	Fri	0815	**Discussion SEMINAR (Crosby):** Can we demonstrate any relationship between UV photochemical change and UV coral reef biology?
7/18	Mon	0815	**Lesser:** Simulation of enhanced UV resulting from ozone depletion
7/19	Tue	0815	**Ondrusek/Lesser:** UV Blockers (Mycosporine-like Amino Acids)
7/20	Wed	0815	**Kinzie/Lesser: Part I** - UV and plant response, unicellular algae
7/21	Th	0815	**Lesser/Kinzie: Part II** - UV and plant response, unicellular algae
7/22	Fri	0815	**Discussion SEMINAR (Lesser):** **1)**How is an action spectrum determined? **2)**What does it tell us.
7/25	Mon	0815	Discussion of research projects
7/26	Tue	0815	**Krupp/Gulko:** UV and Coral Reproduction
7/27	Wed	0815	**Shashar:** UV and visual response: an overview
7/28	Th	0815	Student presentations of current research
7/29	Fri	0815	**Discussion SEMINAR (Kinzie):** **1)**Does UV impact primary production on coral reefs? **2)**What is the evidence?
8/3	8/4	8/5	All students participate in the UV workshop at East-West Center

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APPENDIX II:
ULTRAVIOLET RADIATION IN TROPICAL COASTAL ECOSYSTEMS
Workshop Schedule

3-5 August 1994
East-West Center, Jefferson Hall, Pacific Room
Honolulu, Hawaii, USA
Wednesday, August 3, 1994

Wednesday, August 3, 1994

8:00-10:00 OPENING SESSION. Dr. Paul L. Jokiel (moderator).
- Introductory Remarks.
- David Mauzerall - Ultraviolet Radiation and the Origin of Life.
- Michael Lesser - Summary of Previous UV workshops.
- Discussion.

10:00 - 10:15 BREAK

10:15-12:00. PHOTOCHEMISTRY OF UV. Dr. Donald Crosby (moderator).
- Don Crosby - UV Actinometry.
- Curtis Suttle - Bacteriophage Dosimeter.
- Rita Peachey - UV Phototoxicity in Coral Reef Biota.
- Glenn Miller - Principles of UV photoreaction on Surfaces: Implications for Coral Reef Biologists.
- Discussion

12:00-13:00 Lunch

13:00 -15:00 UV INSTRUMENTATION AND HARDWARE: Dr. Michael Lesser (moderator).
- Michael Lesser - General Overview of Instrumentation, Experimental Methods, Penetration of UV Into Natural Waters
- Alan Teramura - Considerations When Using Artificial Lamps to Supplement UV-B Radiation.
- John Morrow - Scanning Spectroradiometers.
- Discussion.

15:00 - 15:15 BREAK

15:15 - 17:00 REMOTE SENSING, GROUND TRUTH, MONITORING
- Anatoli Gitelson and Yossi Yacobi - Remote Assessment of Chlorophyll Concentration in Productive Waters.
- Karen Patterson - Possibilities of Using Satellite Remote Sensing to Calculate UV Transparency of Waters Over Coral Reefs and Other UV-Related Processes
- Discussion

17:00 Pau Hana (End work day)

Thursday, August 4, 1994.

8:00 - 10:00 BIOLOGICAL RESPONSE TO UV. Dr. Robert Kinzie (moderator)

Robert Kinzie - UV and a Hawaiian High Altitude Aquatic Ecosystem.

Michael Ondrusek, Ania Banaszak, Ilsa Kuffner - UV Blockers

Dr. Dave Krupp, Andrew Baker, Dave Gulko - Coral Reproduction and UV.

Discussion

10:00 - 10:15 BREAK

Peterson *et al.* (presented by Karen Patterson) - A Biological Weighing Function for Phytoplankton Growth Inhibition by UV Based on Growth Responses of Zooxanthellae Cultured Under Various UV conditions.

Satoru Taguchi - UV Damage and Repair in Phytoplankton.

Hiroaki Saito -Effect of UV-B on the Reproduction of Marine Copepods: Hatching Rate of *Paracalanus* sp.

Marjorie Reaka-Kudla - The Relative Effects of Temperature and UVB on Different Components of the Caribbean Reef Community.

Discussion

12:00-13:00 Lunch.

WORKING SESSIONS (moderated by Dr. Zvy Dubinsky and Dr. Paul Jokiel).

Previous sessions have described the "state of the art". The Working Sessions served as a process to describe a UV research program for the future and the role of HIMB (and others) in implementing this program.

The purpose of these sessions was:

1. Define and prioritize research questions.
2. Develop a plan to answer questions.
3. Identify measurement problems, means of resolving problems.

13:00 - 15:00 WORKING SESSION I

Group Development of Conclusions, Recommendations In Each Area.

15:00- 15:15 Break

15:15- 17:00 WORKING SESSION II

Group Development of Conclusions, Recommendations In Each Area.

17:00 Pau Hana.

Friday, August 5, 1994

8:00 -10:00 PHOTOMETRIC MEASUREMENTS AND VISUALIZATION OF THE UV WORLD. THE STATE OF THE ART. Dr. George Losey (moderator).

William McFarland - UV Vision in Marine Fishes.

Craig Hawryshyn - UV Vision in Fish: Strategies for Revealing Receptor Design and Performance.

Tom Cronin - UV Vision in Marine Invertebrates.

Discussion.

10:00 - 10:15 Break

Ellis Loew - UV visualization of Zooplankton: Design and Implementation of Camera Systems.

Diane Fiore - Optical Monitoring of UV in the Environment by Use of Videography.

Discussion.

12:00 -13:00 Lunch.

13:00 -15:00 WORKING SESSION I. Dr. George Losey (moderator). Group Discussion of Research Needs, Design Criteria and Engineering Factors for UV Videography Including Video Imaging, Characterization of the Spectral Composition of Objects and the Environment and an Imaging "Spotmeter".

15:00 -15:15 Break

15:15 - 17:00 WORKING SESSION II. Dr. George Losey (moderator). Group Work to Establish Final Specifications and Future Research Directions.

17:00 Pau Hana

APPENDIX III: UV Measurement Instrumentation Comparison Chart.

The following is an unedited compilation of feedback on various measurement tools used by various scientists who study UV in the marine environment and who attended the Measurement of Ultraviolet Radiation in Tropical Coastal Ecosystems Workshop.

Name of Instrument	Type	Manufacturer	Measurement Units	Wavelength Measurement Range	Time Required	Size/ Weight	Band Width	Estimated Cost	Peripherals, Accessories Required	Contact Researcher
OSMA Spectroradiometer	Radiometer	Princeton Instruments	Photon counts	230 - 800 nm	16 msec per scan	2' x 2' x 2'	Narrow ± 2nm	@$25,000	-Portable computer with interface -Dry air source & water for cooling	Dr. William McFarland, Wrigley Marine Science Center
UW/800 Spectroradiometer	Radiometer	LICOR	=moles/cm2 /nm	300 - 850 nm	45 seconds per scan	Cylinder 30cmx 30cm, 30 lbs	Broad	@$19,000 per unit		Dr. Craig Hawryshyn, University of Victoria
IL-1700	Radiometer	International Light	Variable, Operator-specified	Dependent on sensors installed	Real time	30cm x 20cm x 10cm; 29 lbs	Inter-mediate	$3000 to $4000		Andrew Baker, University of Miami
LI-1800	Radiometer	Biospherical Instruments							LI-1800 Integrating Sphere	Dr. Anatoli Gitelson, Ben Gurion University, ISREAL
UDT PIN-10 DP/SB	Radiometer	United Detector Technology	Variable	<300 - >1000nm	Real time	Diode - 1" cube, Amplifier 4" cube	Depends on filter	@$300 with amplifier	Voltmeter or A/D converter	Dr. Tom Cronin, University of Maryland
Actinometer	Chemical	Self-prepared	Einsteins or =W/cm2	290 - 380 nm	15+ minutes per sample	<10cm long, <50g	Broad	<$0.50/ sample	- Transparent container - Spectrophotometer or HPLC	Dr. Don Crosby, UC-Davis
Vironometer	Biological	Self-prepared	Virus Destruction Rate	@290 - 420 nm	24 hrs for analysis; time scales from hrs to days.	Variable; bunch of plastic bags.	Broad	<$0.50/ sample, plus cost of doing assay	-Autoclave -Pipettors	Dr. Curtis Suttle, University of Texas

Name of Instrument	Can Action Spectra be Acquired?	Affected by Reflectance Albedo etc.?	Special Features	Recommended Fields of Study	Major Advantages	Major Disadvantages	NOTE:	Reference for Method
OSMA Spectroradiometer	No	Yes	Very high sensitivity not present in standard spectro-radiometers	General Spectroradiometry				
UW/800 Spectroradiometer	Yes	Yes	Self calibration with LICOR Calibrator	-Spectral scans -Polarization work	• Complete, waterproof package. • Can be preprogrammed for unattended datalogging.	• Length of time for each scan. • No surface measurements at the same time as underwater ones. • Very heavy. • Limited dynamic range.		A. Hendry & C. Hawryshyn (1992). *J. Exp. Biol.* Novales-Flamarique & Hawryshyn (1993). *Can. J. Aq. Sci.*
IL-1700	No	Yes	Can be remotely accessed from phone lines.	- Measurement of UV at defined depths, under specified environmental conditions. - Laboratory work involving aquatic UV penetration.	• Programmable to any units. • Wide variety of sensors available. • Portable • Simultaneous measurements	• Not ideal for field work, since main unit must be protected from sunlight over long periods of time. • Cannot produce spectra. • Lower accuracy.	Can purchase custom detectors for "effective irradiance" in order to measure specified action spectra.	Glynn *et al.* (1992) *Proc. 7th Internat'l Coral Reef Symposium* 1: 27 - 37.
LI-1800		Yes		-Reflectance Measurements -Underwater Down- and Upwelling Irradiances.				
UDT PIN-10 DP/SB	No			Calibration of Light Sources	• Small • Stable • Inexpensive	• Requires custom underwater housing. • Requires filtering for field use.		
Actinometer	No	Yes	Basically a photon-counter in a specified wavelength range.	Underwater and Terrestrial UV Measurement	• Provides both Intensity & Integral. • Can measure small spatial scales. • Inexpensive and portable. • Fits many configurations.	• Still under development. • Readings not instantaneous. • Requires analytical equipment. • Not highly wavelength specific.	Actinometer must be initially calibrated against a spectro-radiometer.	Dulin & Mill (1982) *Environ. Sci. & Technol.* 16:815 Calvert & Pitts "Photochemistry"
Vironometer	Yes	Yes	Has biological relevance.	Interpreting UV Doses on Small Spatial Scales	• Inexpensive & portable. • Response is linear to dose. • Fits many configurations.	• Measurement is not instantaneous. • Some need for training in growing bacteria and plaque assays.	Needs to be calibrated with other dosimeters.	Suttle & Chen (1992). *Appl. Envir. Microbiol.* 58(11): 3721 - 3729.

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