THE EFFECTS OF ULTRAVIOLET RADIATION ON REEF CORALS AND THE
SUN-SCREENING ROLE OF MYCOSPORINE-LIKE AMINO ACIDS

A DISSERTATION SUBMITTED TO THE GRADUATE DIVISION OF THE
UNIVERSITY OF HAWAI‘I IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY
IN
ZOLOGY (MARINE BIOLOGY)
DECEMBER 1999

By
Ilisa Boysen Kuffner

Dissertation Committee:

Paul L. Jokiel, Chairperson
Evelyn Cox
Robert A. Kinzie, III
Margaret McFall-Ngai
Celia M. Smith
We certify that we have read this dissertation and that, in our opinion, it is satisfactory in scope and quality as a dissertation for the degree of Doctor of Philosophy in Zoology (Marine Biology).

DISSERTATION COMMITTEE

Chairperson
ACKNOWLEDGEMENTS

I would like to thank the wonderful, patient people who helped me in the field: David Bybee, Stuart Field, Erik Kuffner, Elin Kuffner, Steve Kolinski, Amy Lacks, Austin Murai, Ku'ulei Rodgers, Ann Tarrant, Lloyd Waterai, Joanne Wilson, and Brent Womersley. For help with experimental design and methods, thanks go to: Ania Banaszak, Andrea Grottoli, Michael Lesser, Fran Marubini, Kirsten Michalek-Wagner, Stuart Newman, Mike Ondrusek, Jodi Schwarz and Andy Taylor. Walt Dunlap’s hospitality and patience allowed for the production of MAA standards at the Australia Institute of Marine Science. Bob Bidigare generously supplied the photosynthetic pigment standards. Celia Smith and Bob Bidigare graciously allowed me to analyze my samples on their HPLC systems.

For funding, I would like to thank the Lerner-Gray Fund for Marine Research, American Museum of Natural history and the Edmondson Grants-In-Aid for Research, University of Hawaii.

I would also like to thank my committee, Paul Jokiel, Robert Kinzie, Fenny Cox, Celia Smith and Margaret McFall-Ngai, for the inspiration and guidance they have given me towards becoming a marine biologist of my own merit.

Thanks also to my parents, David and Kathleen Kuffner, for all their support through this long journey of education.
ABSTRACT

Shallow-dwelling scleractinian corals live in high irradiance environments where they are exposed to large fluxes of ultraviolet radiation (UVR, 280 – 400 nm). A suite of UV-aborbing compounds, know as mycosporine-like amino acids, is found within the tissues of coral-algal symbioses and may perform a sun-screening role. The seasonal variation in MAA concentration was investigated for two corals in Kaneohe Bay, Hawai‘i, *Porites compressa* and *Montipora verrucosa*. Regressions of MAA concentration and the amount of UVR measured prior to collection date were not significant for total MAA concentration of either species. However, individual MAAs, shinorine in *P. compressa* and palythene in *M. verrucosa*, did show significant correlation with UVR.

The effects of UVR and water motion on *Porites compressa* were investigated in a flume and in the field. Exposure to ambient UVR was the most important factor tested in determining the concentration of MAAs in the tissues of *P. compressa*. Water motion also positively affected the concentration of MAAs, but only in the presence of UVR. When UVR was screened from the corals' environment, the tissue concentration of MAAs slowly decreased over time (approximately 2.5 to 5% per week) regardless of water motion.

The effect of UVR on coral planulae was investigated in field experiments with *Pocillopora damicornis*. Larvae were taken from four different source adults: those from <0.5 m, those from 3 m, those incubated in the absence of UVR for two months, and those incubated in ambient UVR for two months. Deep larvae and larvae from adults
incubated in the absence of UVR had roughly half the amount of MAAs found in the shallow larvae and the larvae from adults in ambient UVR. Origin of larvae was not a significant factor in determining larval survival or recruitment success. UVR, however, was important in determining recruitment rate. Larvae were less likely to recruit to the settlement tile in the presence of ambient UVR than in treatments where the UVR was screened out.
# TABLE OF CONTENTS

**ACKNOWLEDGEMENTS** ................................................................. iii

**ABSTRACT** ................................................................................. iv

**LIST OF TABLES** .......................................................................... x

**LIST OF FIGURES** ....................................................................... xi

**CHAPTER 1: GENERAL INTRODUCTION** ........................................... 1

  **BACKGROUND** ........................................................................... 1

  **THE UVR ENVIRONMENT OF KANEHOE BAY** ................................. 7

  **OBJECTIVES OF THE STUDY** ...................................................... 15

**CHAPTER 2: THE ANNUAL CYCLE OF UV-ABSORBING COMPOUNDS AND PHOTOSYNTHETIC PIGMENTS IN TWO SPECIES OF CORAL IN HAWAI‘I** ........................................................................ 18

  **INTRODUCTION** ......................................................................... 18

  **MATERIALS AND METHODS** ..................................................... 21

    - Sample collection ........................................................................ 21
    - Ultraviolet radiation measurement ........................................... 21
    - Sample preparation ..................................................................... 22
    - HPLC analysis of mycosporine-like amino acids ......................... 22
    - HPLC analysis of photosynthetic pigments ............................... 23
    - Statistical analysis .................................................................... 24

  **RESULTS** .................................................................................. 26

    - Annual trend in ultraviolet radiation .................................... 26
    - Mycosporine-like amino acids ................................................. 32
    - Photosynthetic pigments ......................................................... 43
CHAPTER 5: THE EFFECTS OF ULTRAVIOLET RADIATION ON LARVAL RECRUITMENT OF THE REEF CORAL, *Pocillopora damicornis* Linnaeus

INTRODUCTION ................................................................. 117

MATERIALS AND METHODS ............................................. 119

Collection and maintenance of corals ................................. 119
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1 Surface irradiance in winter and summer</td>
<td>8</td>
</tr>
<tr>
<td>1.2 Surface irradiance under sunny and cloudy conditions</td>
<td>8</td>
</tr>
<tr>
<td>1.3 Vertical attenuation coefficients for tropical waters around the world</td>
<td>14</td>
</tr>
<tr>
<td>2.1 Surface irradiance in winter and summer</td>
<td>30</td>
</tr>
<tr>
<td>2.2 Regression analyses for MAAs and ultraviolet radiation in corals</td>
<td>36</td>
</tr>
<tr>
<td>2.3 Regression analyses for photosynthetic pigments and solstice days in coral</td>
<td>46</td>
</tr>
<tr>
<td>3.1 ANOVA of water motion and UVR effects on <em>Porites compressa</em></td>
<td>71</td>
</tr>
<tr>
<td>4.1 ANOVA of water motion and UVR effects on <em>P. compressa</em> MAAs</td>
<td>99</td>
</tr>
<tr>
<td>4.2 ANOVA of water motion and UVR effects on <em>P. compressa</em> pigments</td>
<td>103</td>
</tr>
<tr>
<td>5.1 Effects of UVR and origin on Pocillopora damicornis larvae</td>
<td>131</td>
</tr>
<tr>
<td>5.2 ANOVA of UVR and origin effects on <em>Pocillopora damicornis</em> larvae</td>
<td>134</td>
</tr>
<tr>
<td>5.3 MAA concentrations in <em>Pocillopora damicornis</em> adults and larvae</td>
<td>136</td>
</tr>
</tbody>
</table>
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>Surface spectral irradiance in winter and summer.</td>
<td>10</td>
</tr>
<tr>
<td>1.2</td>
<td>Surface spectral irradiance under cloudy and clear conditions</td>
<td>12</td>
</tr>
<tr>
<td>2.1</td>
<td>Seasonal cycle in surface ultraviolet radiation</td>
<td>27</td>
</tr>
<tr>
<td>2.2</td>
<td>Surface spectral irradiance in winter and summer</td>
<td>29</td>
</tr>
<tr>
<td>2.3</td>
<td>HPLC chromatogram of MAAs in <em>Porites compressa</em></td>
<td>33</td>
</tr>
<tr>
<td>2.4</td>
<td>HPLC chromatograms of MAAs in <em>Montipora verrucosa</em></td>
<td>35</td>
</tr>
<tr>
<td>2.5</td>
<td>Time course of MAA concentrations in the tissues of <em>Porites compressa</em></td>
<td>38</td>
</tr>
<tr>
<td>2.6</td>
<td>Time course of MAA concentrations in the tissues of <em>Montipora verrucosa</em></td>
<td>40</td>
</tr>
<tr>
<td>2.7</td>
<td>Time course of total MAAs in <em>Porites compressa</em> and <em>Montipora verrucosa</em></td>
<td>42</td>
</tr>
<tr>
<td>2.8</td>
<td>HPLC chromatogram of photosynthetic pigments in <em>Porites compressa</em></td>
<td>45</td>
</tr>
<tr>
<td>2.9</td>
<td>Time course of photosynthetic pigments in <em>Porites compressa</em></td>
<td>48</td>
</tr>
<tr>
<td>2.10</td>
<td>Time course of photosynthetic pigments in <em>Montipora verrucosa</em></td>
<td>50</td>
</tr>
<tr>
<td>2.11</td>
<td>Time course of total pigments in <em>P. compressa</em> and <em>M. verrucosa</em></td>
<td>52</td>
</tr>
<tr>
<td>3.1</td>
<td>MAA concentration vs. time with flume treatment in <em>P. compressa</em></td>
<td>73</td>
</tr>
<tr>
<td>3.2</td>
<td>Chlorophyll a vs. time with flume treatment in <em>P. compressa</em></td>
<td>76</td>
</tr>
<tr>
<td>3.3</td>
<td>Calcification rate vs. time with flume treatment in <em>P. compressa</em></td>
<td>78</td>
</tr>
<tr>
<td>4.1</td>
<td>MAA concentration vs. time with field treatment in <em>P. compressa</em></td>
<td>101</td>
</tr>
<tr>
<td>4.2</td>
<td>Total pigments vs. time with field treatment in <em>P. compressa</em></td>
<td>105</td>
</tr>
<tr>
<td>4.3</td>
<td>Pigments vs. time with field treatment in <em>P. compressa</em></td>
<td>107</td>
</tr>
<tr>
<td>4.4</td>
<td>Calcification rate vs. time with field treatment in <em>P. compressa</em></td>
<td>110</td>
</tr>
<tr>
<td>5.1</td>
<td>Larval recruitment chamber</td>
<td>121</td>
</tr>
<tr>
<td>5.2</td>
<td>Spectral irradiance within UVO and UVT recruitment chambers</td>
<td>130</td>
</tr>
<tr>
<td>5.3</td>
<td>Survival and recruitment of <em>Pocillopora damicornis</em> larvae</td>
<td>133</td>
</tr>
</tbody>
</table>
CHAPTER 1: GENERAL INTRODUCTION

BACKGROUND

Ultraviolet radiation (UVR, 280 - 400 nm) is an important physical parameter affecting life on Earth. Important biological molecules, such as DNA, RNA, and proteins, absorb and are damaged by UVR (Holm-Hansen et al. 1993). Biological organisms evolved in the presence UVR, and have developed protective and repair mechanisms to mitigate its effects (Calkins and Thordardottir 1980; Mitchell and Karentz 1993; Cockell and Knowland 1999).

The intensity of UVR reaching an organism’s habitat is mainly a function of the zenith angle of the sun and the concentration of ozone in the stratosphere. The solar zenith angle is determined by latitude, season, and time of day. The stratospheric concentration of ozone, which is the primary component of the atmosphere responsible for the absorption of UVR <330 nm (Hader and Worrest 1991), is positively correlated with latitude. The combination of lower zenith angles and lower concentrations of ozone establishes that UVR flux is generally greatest at the equator and decreases with increasing latitude (Holm-Hansen et al. 1993).

Aquatic organisms do not escape the effects of UVR. Most open-ocean and coastal waters in the tropics are highly transparent to UVR that can penetrate the water column to depths of 30 m (Jerlov 1950; Smith and Baker 1979). The condition of the water column is very important in determining the exposure of aquatic organisms to solar radiation. The absorptive properties of the water alter the quantity and quality of solar
radiation by scattering or absorbing photons on a wavelength-dependent basis (Kirk 1994). Light absorption in the water column is attributable to four main components: the water itself, dissolved yellow pigments (gilvin), photosynthetic organisms, and non-living particulate matter (tripton) (Kirk 1994). The absorption spectrum of gilvin is strong in the ultraviolet.

The accumulation of chlorofluorocarbons (CFCs) within the stratosphere has been linked to the degradation of ozone (Farman et al. 1985; Anderson et al. 1991), leading to the annual formation of the “ozone hole” over Antarctica in the austral spring (Schoeberl and Hartmann 1991). Two-fold increases in UVB have been measured at these high latitudes during ozone hole events (Frederick and Snell 1988; Kerr and McElroy 1993). The effects of enhanced UVR on aquatic ecosystems in this region have been intensely investigated, leading researchers to conclude that decreased primary productivity will probably result, with cascading effects to all trophic-levels (Hader and Worrest 1991; Bothwell et al. 1994). The fact remains however that tropical regions receive the highest levels of UVR found at sea level on Earth. Even during a well-developed ozone hole on the austral spring equinox, UVB radiation levels in the tropics are approximately an order of magnitude higher than those reaching the Earth’s surface under the ozone hole (Holm-Hansen et al. 1993). Studying the adaptive mechanisms employed by organisms in places where UVR is near the global extreme may prove quite useful when considered in concert with ozone hole-related research. Specifically, it would be instrumental to estimate the species-specific costs associated with mitigating the effects of ambient UVR in the tropics before making predictions regarding the effects of excess UVR at high latitudes.
Coral reef ecosystems are typical of tropical, high-irradiance environments. One of the first researchers to recognize the importance of UVR on coral reefs, Jokiel (1980) demonstrated the ecological significance of UVR in determining community structure. In his study, relocating cryptic animals to a high-irradiance environment caused high mortality, except when UVR was screened out using filters transparent to visible wavelengths only. Jokiel’s work indicated that organisms inhabit species-specific ranges in UVR exposure, bringing to view a new niche dimension not previously recognized on coral reefs. The next question to ask was how do organisms that live in high UVR environments accomplish this? Some sun-adapted members of the community have evolved structural protection such as shells and chitinous exoskeletons that can shield UVR. The scleractinian corals represent taxa that must employ other means of protection, for the zooxanthellae resident within their tissues require visible light for photosynthesis.

A family of UV-absorbing compounds was first extracted from coral tissue by Shibata (1969) and termed the ‘S 320’ complex due to the compounds’ absorptive properties in the 310 to 380 nm range of the solar spectrum. These compounds are now referred to as mycosporine-like amino acids (MAAs), due to their structural similarity to the mycosporines found in sporulating fungi (Leach 1965; Favre-Bonvin et al. 1976). MAAs have been isolated from the tissues of marine organisms across many phyla of the animal kingdom (Dunlap and Chalker 1986; Karentz et al. 1991), as well as phytoplankton (Carreto et al. 1990; Lesser 1996), macroalgae (Beach et al. 1997; Banaszak et al. 1998), and cyanobacteria (Garcia-Pichel and Castenholz 1993). There are presently 19 known MAAs, all sharing the same basic molecular structure of a
cyclohexenone or cyclohexenimine ring conjugated with the nitrogenous component of an amino acid. They are water-soluble and probably exist in ionic form in cell cytosol (W.C. Dunlap, pers. comm.). The single known biosynthetic source for MAAs is the shikimate-acid pathway, only found in photosynthetic bacteria, marine algae and fungi (Bentley 1990). Given the plethora of non-photosynthetic organisms that have been found to contain MAAs, it was hypothesized that the compounds are accumulated through diet. This hypothesis has recently been supported in a number of studies involving organisms of different phyla: the sea urchin, *Strongylocentrotus droebachiensis* (Adams and Shick 1996), the sea hare, *Aplysia dactylomela* (Carefoot et al. 1998), and the teleost, *Oryzias latipes* (Mason et al. 1998). Corals represent a complicated case, in that they may acquire MAAs from the zooxanthellae, from diet, or both. The non-symbiotic scleractinian, *Tubastrea coccina*, has been found to contain MAA concentrations comparable to symbiotic species of coral (Banaszak et al. 1998), suggesting that this species may obtain MAAs from its diet.

Given the ubiquity of MAAs throughout the marine ecosystem, their absorbance properties, and a well-established inverse relationship between depth and MAA concentration (Maragos 1972; Dunlap et al. 1986; Shick et al. 1995; Banaszak et al. 1998), researchers postulated a photoprotective role (Carreto et al. 1990; Karentz et al. 1991; Garcia-Pichel et al. 1993). Evidence in support of this putative protective role has been extensive but mostly indirect. Experimental evidence is only now beginning to appear in the literature (see review by Dunlap and Shick 1998). Of particular significance is a study conducted by Shick et al. (1995), which established the connection between MAA concentration and the photosynthetic performance of the hermatypic
coral, *Acropora microphthalma*, on the Great Barrier Reef. Corals from shallow environments were unaffected by UVR when placed in an underwater respirometer at 1 m depth, whereas photosynthesis in corals from 20 and 30 m depth showed 30 and 38% inhibition, respectively, when brought to 1 m depth. The authors attributed the difference in photosynthesis to the five-fold higher concentrations of MAAs in the shallow corals compared to their deep counterparts.

In order for a sun-screening role to be ascribed to MAAs, it is important to establish that UVR is the cue that determines MAA concentration (Cockell and Knowland 1999), and to pinpoint other factors that may take part in controlling the synthesis and accumulation of MAAs. UVR attenuates exponentially with water column depth (Jerlov 1950; Fleischmann 1989), but so do photosynthetically active radiation (PAR) (Kirk 1994) and water motion (Helmuth et al. 1997b). Water motion is positively correlated to photosynthetic rate in coral (Dennison and Barnes 1988; Patterson et al. 1991; Lesser et al. 1994), coral growth rate (Jokiel 1978), and phosphate uptake rate by coral reefs (Atkinson and Bilger 1992). There is general agreement that these phenomena result from the reduction in the thickness of the diffusion boundary layer (DBL), allowing higher exchange rates of gaseous substrates and waste products utilized in photosynthesis and other metabolic activities. Thus, there is reason to suspect that MAA concentration may be affected by water motion, as MAA production is undoubtedly related to photosynthesis and/or metabolism.

The importance of MAAs in the coral-algal symbiosis must be assessed on various temporal scales. If MAAs are important for UVR protection, concentration of MAAs should correlate with the dose of UVR received throughout the year. Also, the
significance of the compounds in the complete life cycle of a coral must be investigated. Coral larvae are either released into the water after being brooded within the adult polyp, or result from external fertilization of gametes simultaneously projected into the water column (for review see Richmond and Hunter 1990). Regardless of their origin, larvae may spend an appreciable amount of time near the surface of the water before settling (Richmond 1987), and thus may be exposed to higher levels of UVR at this stage of life than as adults. The significance of MAAs and UVR to coral larvae has been investigated in only two studies so far (Baker 1995; Gleason and Wellington 1995), both of which suggest that MAAs do perform a sun-screening role in this part of the life cycle.
THE UVR ENVIRONMENT OF KANEHOE BAY

Characterization of the light environment in and around Kaneohe Bay was an important aspect of this study. The purpose of obtaining these data was to establish the range of UVR over which organisms within Kaneohe Bay are exposed. Measurements were made throughout the year with a LiCor® LI-1800UW scanning spectroradiometer (LiCor®, Lincoln, Nebraska), both at the surface and within the water column. Spectroradiometric scans were taken on cloudless days in summer and winter in order to document temporal changes in the quantity and quality of solar radiation. Organisms at the surface of Kaneohe Bay in the summer at noon are exposed to UVR irradiance rates nearly double those in the winter at noon (Fig. 1.1). Integration of the data revealed that UVR attenuates more than PAR on an annual cycle (Table 1.1). The data also indicated that UVB irradiance attenuates more than UVA from summer to winter (Table 1.1).

Cloud cover had a dramatic effect on spectral irradiance, particularly in the visible wavelengths (Fig. 1.2). Two scans were taken within 20 minutes of each other, the first under a clear sky and the second after a cumulus cloud had totally obscured the solar disc (i.e., all light was diffuse). These scans demonstrate that this type of cloud diminishes PAR to a greater degree than UVR; 38% of the UVR penetrated the cloud, while only 27% of the PAR remained (Table 1.2). Changes in spectral irradiance due to cloud cover are more substantial than changes due to annual variation.
Table 1.1. Seasonal comparison of surface irradiance on two cloudless days measured within 20 minutes of maximum sun altitude, and on one day before and after the emergence of a cumulus cloud. Integrated values of UVB (300-320 nm), UVA (320 – 400 nm), UVR (300-400 nm) and PAR (400-700) are reported in W·m⁻²·nm⁻¹. Percent of summer value calculated by dividing the winter value by the summer value x 100.

<table>
<thead>
<tr>
<th>Waveband</th>
<th>July 2, 1998</th>
<th>Jan 7, 1998</th>
<th>% of summer value</th>
</tr>
</thead>
<tbody>
<tr>
<td>UVB</td>
<td>4.02</td>
<td>2.21</td>
<td>55.0%</td>
</tr>
<tr>
<td>UVA</td>
<td>59.5</td>
<td>39.6</td>
<td>66.6%</td>
</tr>
<tr>
<td>UVR</td>
<td>63.2</td>
<td>41.5</td>
<td>65.7%</td>
</tr>
<tr>
<td>PAR</td>
<td>471</td>
<td>358</td>
<td>76.0%</td>
</tr>
<tr>
<td>UVR:PAR</td>
<td>0.134</td>
<td>0.116</td>
<td></td>
</tr>
<tr>
<td>UVB:UVA</td>
<td>0.068</td>
<td>0.056</td>
<td></td>
</tr>
</tbody>
</table>

Table 1.2. Comparison of surface irradiance under cloudy and clear conditions. Cloud measurement was made just following the emergence of a cumulus cloud. Integrated values of UVB (300-320 nm), UVA (320 – 400), UVR (300-400 nm) and PAR (400-700) are reported in W·m⁻²·nm⁻¹. Percent of sun value calculated by dividing the cloud value by the sun value x 100.

<table>
<thead>
<tr>
<th>Waveband</th>
<th>Aug 8, 1999 Sun</th>
<th>Aug 8, 1999 Cloud</th>
<th>% of sun value</th>
</tr>
</thead>
<tbody>
<tr>
<td>UVB</td>
<td>3.80</td>
<td>1.45</td>
<td>38.2%</td>
</tr>
<tr>
<td>UVA</td>
<td>60.2</td>
<td>19.8</td>
<td>32.9%</td>
</tr>
<tr>
<td>UVR</td>
<td>64.0</td>
<td>21.3</td>
<td>33.3%</td>
</tr>
<tr>
<td>PAR</td>
<td>513</td>
<td>138</td>
<td>26.9%</td>
</tr>
<tr>
<td>UVR:PAR</td>
<td>0.125</td>
<td>0.154</td>
<td></td>
</tr>
<tr>
<td>UVB:UVA</td>
<td>0.063</td>
<td>0.073</td>
<td></td>
</tr>
</tbody>
</table>
Figure 1.1. Kaneohe Bay, Oahu. Spectral irradiance (Watts·m⁻¹·nm⁻¹) as a function of wavelength (nm) as measured on two days, January 7 and July 2, 1998, at 12:13 and 12:12 P.M., respectively. No clouds were visible when the scans were taken.
Spectral Irradiance (W m\(^{-2}\) nm\(^{-1}\))

Wavelength (nm)

- July 2 (12:12 PM)
- January 7 (12:13 PM)
Figure 1.2. Kaneohe Bay, Oahu. Spectral irradiance (Watts·m\(^{-1}\)·nm\(^{-1}\)) as a function of wavelength (nm) as measured before (12:48 P.M.) and after (1:08 P.M.) a cumulus cloud emerged. The sun was not visible through this cloud (i.e., all light was diffuse).
Attenuation of solar irradiance through the water column is also of importance to aquatic organisms within shallow waters. The vertical attenuation coefficient \( K_d \) was calculated from field measurements at several sites inside and outside of Kaneohe Bay (Table 1.3). The values for \( K_d \) obtained within the Bay on three different days at two different locations were in close agreement, averaging 0.26 m\(^{-1}\). The estimated \( K_d \) at the Moku Manu site is comparable to those reported for other tropical waters around the world (Table 1.3). Attenuation of all wavebands was much higher within the Bay than outside the Bay. Inside the Bay, the depth at which 10% of the sub-surface UVR remained was 4.6 m. Outside the Bay, this depth was 28.7 m. The optical quality of the water column differs greatly between these two environments, and undoubtedly plays a large role in determining community structure at each locale.
Table 1.3. Vertical attenuation coefficients for downward irradiance (K_d) for integrated UVB irradiance (300-320 nm), UVR (300-400 nm) and PAR (400-700) in tropical waters. n/a = not available.

<table>
<thead>
<tr>
<th>Location</th>
<th>K_d (UVB) (m⁻¹)</th>
<th>K_d (UVR) (m⁻¹)</th>
<th>K_d (PAR) (m⁻¹)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kaneohe Bay, Oahu</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sliver Reef 7/3/94</td>
<td>0.87</td>
<td>0.50</td>
<td>0.28</td>
<td>Present study</td>
</tr>
<tr>
<td>Point Reef 3/9/98</td>
<td>0.62</td>
<td>0.32</td>
<td>0.22</td>
<td>Present study</td>
</tr>
<tr>
<td>Point Reef 6/13/99</td>
<td>n/a</td>
<td>n/a</td>
<td>0.29</td>
<td>Present study</td>
</tr>
<tr>
<td>Off shore, Oahu</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moku Manu</td>
<td>0.18</td>
<td>0.08</td>
<td>0.05</td>
<td>Present study</td>
</tr>
<tr>
<td>Off shore</td>
<td>n/a</td>
<td>n/a</td>
<td>0.03</td>
<td>within Kirk, 1994*</td>
</tr>
<tr>
<td>Sargasso Sea</td>
<td>n/a</td>
<td>n/a</td>
<td>0.03</td>
<td>within Kirk, 1994*</td>
</tr>
<tr>
<td>Carrie Bow Cay, Belize</td>
<td>0.32</td>
<td>0.11</td>
<td>0.08</td>
<td>Banaszak et al., 1998</td>
</tr>
<tr>
<td>Gulf Stream, Bahamas</td>
<td>n/a</td>
<td>n/a</td>
<td>0.08</td>
<td>within Kirk, 1994*</td>
</tr>
</tbody>
</table>

* See Table 6.1, pp. 138 – 143 (Kirk, 1994).
OBJECTIVES OF THE STUDY

The objectives of this study were: 1) to establish natural variability in MAA concentrations throughout the year for two common reef-building corals, 2) to examine the effects of UVR and water motion on the concentration of MAAs in coral, and 3) to examine the effects of UVR and MAA concentration on coral larval recruitment.

The first objective is addressed in Chapter 2, which examined the annual trends in MAA and photosynthetic pigment concentrations in two species of coral. *Porites compressa* and *Montipora verrucosa*, were collected from the Kaneohe Bay reef flat on a monthly basis. Existing high performance liquid chromatography (HPLC) techniques were utilized to quantify the MAAs and the four major photosynthetic pigments: chlorophyll $a$ (chl $a$), chlorophyll $\beta$ (chl $\beta$), peridinin, and diadinoxanthin. Several studies have demonstrated the inverse correlation between coral depth and concentration of MAAs (Maragos 1972; Dunlap et al. 1986; Shick et al. 1995; Banaszak et al. 1998). If the correlation of MAA concentration with depth is a response to incident UVR, then MAA concentration would also be expected to correlate with the annual cycle of UVR. The concentration of photosynthetic pigments in the coral-algal symbiosis should also exhibit annual trends. Photoacclimation by the symbiosis to low light availability has been described along a depth gradient (for review see Falkowski et al. 1990) and with host morphology (Helmuth et al. 1997a), but annual changes in pigment concentration have not been addressed adequately. In many of the photoacclimation studies published so far, chl $a$ was the only photosynthetic pigment quantified, with chl $\beta$ and peridinin measured occasionally (see Table 3 in Kaiser et al. 1993). Only recently have other accessory
pigments been considered in coral research (Helmuth et al. 1997a; Iglesias-Prieto and Trench 1997). In the present study, all of the major pigments were quantified.

The second objective of this study, to determine if UVR and/or water motion affect MAA concentration, is addressed in Chapters 3 and 4. Water motion is an important physical parameter in the marine environment because of its direct influence on uptake rates of metabolically important substrates. Water motion also co-varies with UVR along a depth gradient. In order to quantify the separate and synergistic effects of water motion and UVR on MAA concentration, manipulative studies were required. The effects of UVR and water motion on Porites compressa were tested in two factorial experiments, one in a flume (Chapter 2) and one in the field (Chapter 4). Calcification rate and photosynthetic pigment concentration were also measured in these experiments in order to quantify any metabolic burden of the two factors to the coral host or the zooxanthellae. All four of the major photosynthetic pigments in the field experiment, as well as the MAAs in both experiments, were quantified using HPLC techniques.

Chapter 5 addresses the third objective of this study, examining the effects of UVR and MAA concentration on coral recruitment. The Indo-pacific reef coral, Pocillopora damicornis, was chosen for this study because of the ease in obtaining large numbers of brooded larvae on a reliable basis. This study examined a hypothesis put forth by Gleason and Wellington (1995), that corals from deep habitats have lower reproductive success than shallow conspecifics because the larvae do not have sufficient concentrations of MAAs for protection against UVR. Three factorial experiments were performed using specially designed larval rearing chambers that allowed larvae to be reared at 1 m depth on the reef in conditions of ambient UVR or no UVR.
CHAPTER 2: THE ANNUAL CYCLE OF UV-ABSORBING COMPOUNDS AND PHOTOSYNTHETIC PIGMENTS IN TWO SPECIES OF CORAL IN HAWAI'I

INTRODUCTION

Solar irradiance is an important abiotic factor affecting the ecology of hermatypic scleractinians. The algal symbionts (zooxanthellae) resident in the gastrodermis of the coral animal require access to photosynthetically active radiation (PAR, 400 – 750 nm) for diurnal carbon gain, much of which is exported in support of the symbiosis (Muscatine 1990). Ultraviolet radiation (UVR, 280 - 400 nm) also affects the ecology of corals in shallow water habitats (Jokiel and York 1982; Shick et al. 1996). UVR can penetrate the water column down to 30 m in clear, oligotrophic waters commonly found in the tropics (Jerlov 1950; Smith and Baker 1979). UV-B (280 – 320 nm) radiation is absorbed by and causes damage to nucleic acids and proteins (Holm-Hansen et al. 1993). In addition, the absorption of UVB and UVA (320 – 400 nm) by other cellular components can cause oxidative damage via the production of active oxygen species including singlet oxygen and oxygen, hydroperoxyl, and hydroxyl radicals (Dunlap and Yamamoto 1995).

A suite of UV-absorbing compounds found in the tissues of corals has been intensively investigated for their sun-screening potential (for review see Dunlap and Shick 1998). Known as mycosporine-like amino acids (MAAs), these compounds have been found in orders of magnitude higher concentrations in shallow-dwelling corals.
compared to deep conspecifics (Dunlap et al. 1986; Banaszak et al. 1998). There has been debate over whether these UV-absorbing compounds actually benefit the coral as sunscreens; circumstantial evidence continues to mount in support of this view, but direct evidence is lacking. Several species of coral increase concentrations of MAAs when transplanted from low to high irradiance environments (Scelfo 1986; Gleason and Wellington 1993). The naturally occurring, seasonal variation in MAA concentration in corals has received little attention (except see Drollet et al. 1997), but offers a natural experiment by which we can evaluate acclimation potential and the role of MAAs in UVR protection.

Acclimation by coral-algal symbioses to changes in the ambient PAR environment is most obvious along a depth gradient, typically within 0 to 50 m depth (Chalker and Dunlap 1983; Falkowski et al. 1990; Masuda et al. 1993). The concentration of photosynthetic pigments per unit of coral surface area positively correlates with depth. Shade-acclimated colonies have higher densities of zooxanthellae, or zooxanthellae contain greater amount of pigment per algal cell than light-acclimated colonies (Porter et al. 1984), thereby offsetting decreases in light availability. Recently, changes in zooxanthellar densities were found to correlate with annual solar attenuation (Stimson 1997; Fagoonee et al. 1999). However, neither of these studies quantified the photosynthetic pigments. A clearer understanding of the natural variation in coral pigmentation is imperative, as coral bleaching becomes more frequent and intense (Hoegh-Guldberg 1999).

Tissue concentrations of chl \( a \) are often measured as the sole index of the light-harvesting capacity of the coral-algal symbiosis (see Table 3 in Kaiser et al. 1993), which
in turn is often used as an indicator of the physiological state of the symbiosis. The
historic trend of only measuring chl a could be misleading as other pigments may be
more responsive to changes in the light regime (Chang et al. 1983; Iglesias-Prieto and
Trench 1997). The carotenoids, for example, may perform a photoprotective role by
quenching excess visible light, as demonstrated for the xanthophylls in free-living
dinoflagellates (Demers et al. 1991) and just recently for coral-algal symbioses (Brown et
al. 1999). In dinoflagellates, the light harvesting is accomplished with chl a and the
accessory pigments chl c2 and peridinin (Iglesias-Prieto and Trench 1997). Only recently
have the accessory photosynthetic pigments found in the zooxanthellae been considered
with respect to coral ecology (Fang et al. 1995; Ambarsari et al. 1997; Helmuth et al.
1997a).

The aim of this study was to document the annual cycle in all photosynthetic
pigments and photoprotective compounds in two species of coral in Hawai‘i. The tissue
concentrations of photosynthetic pigments and MAAs were measured using high
performance liquid chromatography (HPLC) for samples of Porites compressa Dana and
Montipora verrucosa Vaughan, 1907 collected on a monthly basis at 1 m depth.
Recording the changes in photosynthetic pigment concentrations throughout the year will
increase our knowledge regarding the acclimation of corals to a dynamic light
environment. Documenting seasonality in MAA concentrations, and correlating these
fluctuations with UVR measured on site, could provide further evidence for the putative
photoprotective role of these compounds.
MATERIALS AND METHODS

Sample collection

Samples were collected monthly from the windward reef flat of Coconut Island, Kaneohe Bay, Hawai‘i (21° 25’N, 157° 45’W) from January 1998 to March 1999. Three small branches of each species, Porites compressa and Montipora verrucosa, were collected on 14 collection dates, each from a different colony at 1 m depth. The probability that a single colony was sampled twice is very low, as the area sampled was approximately 600 m². Samples were immediately transported to a −50°C freezer (in less than five minutes) and stored whole until extracted in methanol for HPLC analysis.

Ultraviolet radiation measurement

Irradiance data were obtained from the weather station at the Hawaii Institute of Marine Biology. UVR (295 - 385 nm) was measured with an Eppley® Ultraviolet Radiometer (Eppley Laboratory Inc., Newport, Rhode Island) in milliwatts per square centimeter (mW cm⁻²). Measurements were taken continuously every two minutes and an average value recorded hourly. Hourly rates were then used to calculate a total dose of UVR for each day in J cm⁻² day⁻¹.

Solar irradiance measurements were also made with a LiCor® LI-1800UW scanning spectroradiometer (LiCor, Lincoln, Nebraska). Spectroradiometric scans were obtained for two cloudless days, one near the winter solstice (January 7, 1998), and one
close to the summer solstice (July 2, 1998). These measurements allowed for the comparison of the quality of surface irradiance during the winter and summer.

Sample preparation

All manipulations were performed in subdued lighting. Each coral was placed in a 50 ml centrifuge tube containing 10 ml of 100% HPLC-grade methanol, and sonicated surrounded by ice water for six minutes. The extracts were then centrifuged at 4°C for two minutes at 1400 x g. The supernatant was decanted and stored at -20°C. Re-extraction occurred twice, adding the new supernatant to the appropriate previous supernatant, resulting in a total extraction volume of 30 ml per coral sample. A 1.2-ml aliquot of the pooled extract was then transferred to an amber HPLC autosampler vial, flushed with pure nitrogen gas, and analyzed for photosynthetic pigments within five hours of extraction. A second aliquot of 1.2 ml was transferred to a microcentrifuge tube and centrifuged at 4000 x g to eliminate particulate matter. The aliquot was then transferred to an amber autosampler vial and stored at -20°C for later analysis of mycosporine-like amino acids. Pigment and MAA concentrations were normalized to surface area for *P. compressa* determined using the aluminum foil technique (Marsh 1970), and dry skeletal mass for *M. verrucosa*.

HPLC analysis of mycosporine-like amino acids

The analysis of MAAs was performed by slightly modifying the procedures described in Dunlap and Chalker (1986) and Shick et al. (1992) on a Shimadzu® HPLC
system. MAAs were separated by reverse-phase isocratic HPLC on a Brownlee® RP-8 column (25 cm, 5 μm) protected with a RP-8 guard, in an aqueous mobile phase of 0.1% acetic acid and 40% methanol. Detection of peaks was via UV absorbance at 313 and 340 nm, and flow rate was 0.8 ml min⁻¹. Identities of peaks were confirmed by co-chromatography with authentic, quantitative standards prepared under the guidance of W.C. Dunlap (Australia Institute of Marine Science). Peaks were integrated using EZ-Chrom™ software, and quantification of the individual MAAs was accomplished using the quantitative standards for calibration.

**HPLC analysis of photosynthetic pigments**

The analysis of the photosynthetic pigments was performed using a slightly modified version of the procedure described in Goericke and Repeta (1993). Pigments were separated by reverse-phase HPLC on a Brownlee® RP-8 (25 cm, 5 μm) column protected by an RP-8 guard using a binary gradient solvent system. Solvents A (MeOH:0.5 N aq. Ammonium acetate, 75:25) and B (MeOH) were programmed on the following gradient (minutes; % solvent A, % solvent B): (0; 100, 0), (20; 0, 100), (25; 0, 100), (33; 100, 0). Detection was at 440 nm, and the flow rate was 1 ml min⁻¹. R. Bidigare (University of Hawaii) generously supplied purified standards of chl c₂, peridinin and diadinoxanthin. Purified chl a was purchased from Aldrich Chemical Company, Milwaukee, Wisconsin. The crystallized standards were dissolved in the appropriate solvent, the absorbance measured at the appropriate wavelength, and the
concentration calculated using the published extinction coefficients for the appropriate solvent.

Statistical analysis

For the dependent variables of total MAAs and each of the individual MAAs, regression analyses were performed with five different independent variables involving the amount of UVR measured on site during the weeks preceding collection of the corals. Five different time periods were defined as follows:

- UVR1 = total UVR measured during the 7-day period prior to collection date
- UVR2 = total UVR measured during the 7-day period two weeks prior to collection date
- UVR3 = total UVR measured during the 14-day period prior to collection date
- UVR4 = total UVR measured during the 21-day period prior to collection date
- UVR5 = total UVR measured during the 14-day period two to three weeks prior to collection date.

Total and individual photosynthetic pigments were regressed with the number of days since or until the nearest winter solstice (December 21). This independent variable was used in lieu of PAR because these data were not available during the collection period because of sensor failure. The ratios of the individual accessory pigments to chl a were also examined as a measure of photoacclimation by the zooxanthellae (e.g., Helmuth et al. 1997a).

In order to reduce the number of regressions performed, the data were first assessed graphically. If no pattern emerged, then the regression was not conducted. If no pattern was apparent for any of the UVR time periods for a particular MAA, then only
the total UVR measured during the 21-day period prior to collection date (UVR4) was
regressed and reported. The assumptions of the regression models were met in all cases
as assessed by examining plots of residuals vs. independent variables, residuals vs.
predicted values, and normal probability plots.
RESULTS

Annual trend in ultraviolet radiation

Integrated values of UVR in J cm\(^{-2}\) day\(^{-1}\) are plotted in Fig 2.1. The three-week period prior to coral collection date with the highest accumulative UVR value was from July 22 to August 11, 1998, with a value of 4229 J cm\(^{-2}\). The lowest corresponding three-week period value of 1650 J cm\(^{-2}\) occurred between November 27 and December 17, 1998. This winter value is 39% of the summer value.

Spectroradiometric measurements for two cloudless days, one near the winter solstice (January 7, 1998), and one close to the summer solstice (July 2, 1998) are graphically compared in Fig 2.2. The scans indicate that different wavelengths do not attenuate to the same extent over an annual cycle. Table 2.1 shows integrated values of irradiance for different wavebands on the two days being compared. These values represent the theoretical difference in UVR between summer and winter because they do not reflect the changes in meteorological events (i.e., cloud cover).
Fig 2.2. Spectral irradiance scans taken at the Hawaii Institute of Marine Biology on January 7, 1998 and July 2, 1998 within 20 minutes of maximum solar altitude.
Table 2.1. Surface irradiance measured on two cloudless days within 20 minutes of maximum sun altitude for integrated UVB (300-320 nm), UVA (320 – 400), UVR (300-400 nm) and PAR (400-700) in W m$^{-2}$ nm$^{-1}$. Percent of summer value calculated by dividing the winter value by the summer value x 100.

<table>
<thead>
<tr>
<th>Waveband</th>
<th>July 2, 1998</th>
<th>Jan 7, 1998</th>
<th>% of summer value</th>
</tr>
</thead>
<tbody>
<tr>
<td>UVB</td>
<td>4.02</td>
<td>2.21</td>
<td>55%</td>
</tr>
<tr>
<td>UVA</td>
<td>59.54</td>
<td>39.55</td>
<td>66%</td>
</tr>
<tr>
<td>UV</td>
<td>63.18</td>
<td>41.54</td>
<td>66%</td>
</tr>
<tr>
<td>PAR</td>
<td>470.95</td>
<td>358.07</td>
<td>76%</td>
</tr>
<tr>
<td>PAR:UV</td>
<td>7.45</td>
<td>8.62</td>
<td></td>
</tr>
<tr>
<td>UVA:UVB</td>
<td>14.8</td>
<td>17.9</td>
<td></td>
</tr>
</tbody>
</table>
Mycosporine-like amino acids

There were three MAAs identified in *P. compressa*: mycosporine-glycine, shinorine and asterina-330 (Fig. 2.3), and five identified in *M. verrucosa*: mycosporine-glycine, shinorine, porphyra-334, palythine and palythene (Fig. 2.4). There was also an unknown peak in the chromatograms of *P. compressa*, but the peak area was typically less than 10% of the total peak area.

Regressing the total amount of UVR during the five time periods with MAA concentrations revealed that only the MAA shinorine in *P. compressa*, and possibly the MAA palythene in *M. verrucosa*, exhibited significant positive seasonal cycles correlated with UVR (Table 2.2) (Figs. 2.5 to 2.7). These relationships were fairly weak, however, with the accumulated UVR measured during the one week prior to collection (UVR1) explaining only 18.5% of the variation in the concentration of shinorine (*p*<0.0045), and 8.7% in palythene (*p*<0.0574). In *M. verrucosa*, no UVR time period explained more than 9% of the variation in any MAA concentration. The total amount of UVR measured in one, two or three weeks prior to collection was not a good predictor of total MAA concentration in either species of coral.

Although the regression model with UVR was not significant, mycosporine-glycine showed a decrease in concentration during the summer months of June, July and August 1998 in *P. compressa* (Table 2.2) (Fig. 2.5). The estimated slope of the regression line was negative (*p*<0.0902) suggesting an inverse relationship between UVR and mycosporine-glycine concentration; however, the $r^2$ value was low ($r^2$ = 0.07).
Fig. 2.3. Representative HPLC chromatogram of mycosporine-like amino acids from 100% methanolic extracts of *Porites compressa*. Detection is at 313 nm.
Fig. 2.4. Representative HPLC chromatogram of mycosporine-like amino acids from 100% methanolic extracts of *Montipora verrucosa*. Detection is at (A) 313 nm and (B) 340 nm.
Absorbance Units

Time (minutes)

Shinorine
Porphyra-334
Palythine
Palythene

Mycosporine-gly
Shinorine
Porphyra-334
Palythine
Table 2.2. Results of regression analysis for mycosporine-like amino acids (MAAs) in the tissues of *Porites compressa* and *Montipora verrucosa* and the amount of ultraviolet radiation (UVR) measured during the weeks prior to collection (see methods section for definitions of the five time periods). P-values < 0.1 are highlighted in bold.

<table>
<thead>
<tr>
<th>Model</th>
<th>$R^2$</th>
<th>F</th>
<th>df</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Porites compressa</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total MAAs = 168.8 - 0.000288(UVR4)</td>
<td>0.000</td>
<td>0.00</td>
<td>38</td>
<td>0.965</td>
</tr>
<tr>
<td>Mycosporine-gly = 66.41 - 0.0210(UVR1)</td>
<td>0.070</td>
<td>3.02</td>
<td>41</td>
<td>0.090</td>
</tr>
<tr>
<td>Mycosporine-gly = 67.62 - 0.00586(UVR4)</td>
<td>0.064</td>
<td>2.51</td>
<td>38</td>
<td>0.121</td>
</tr>
<tr>
<td>Shinorine = 1.77 + 0.0213(UVR1)</td>
<td>0.185</td>
<td>9.08</td>
<td>41</td>
<td>0.005</td>
</tr>
<tr>
<td>Shinorine = 4.65 + 0.0189(UVR2)</td>
<td>0.115</td>
<td>4.80</td>
<td>38</td>
<td>0.035</td>
</tr>
<tr>
<td>Shinorine = 1.71 + 0.0110(UVR3)</td>
<td>0.159</td>
<td>6.98</td>
<td>38</td>
<td>0.012</td>
</tr>
<tr>
<td>Shinorine = 1.67 + 0.0056(UVR4)</td>
<td>0.148</td>
<td>6.41</td>
<td>38</td>
<td>0.016</td>
</tr>
<tr>
<td>Shinorine = 2.39 + 0.0108(UVR5)</td>
<td>0.131</td>
<td>5.56</td>
<td>38</td>
<td>0.024</td>
</tr>
<tr>
<td>Asterina-330 = 99.48 + 0.000011(UVR4)</td>
<td>0.000</td>
<td>0.00</td>
<td>38</td>
<td>0.999</td>
</tr>
<tr>
<td><strong>Montipora verrucosa</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total MAAs = 2112 + 0.151(UVR4)</td>
<td>0.035</td>
<td>1.33</td>
<td>38</td>
<td>0.226</td>
</tr>
<tr>
<td>Mycosporine-gly = 166.8 - 0.00372(UVR4)</td>
<td>0.005</td>
<td>0.18</td>
<td>38</td>
<td>0.676</td>
</tr>
<tr>
<td>Shinorine = 30.22 + 0.00424(UVR4)</td>
<td>0.010</td>
<td>0.37</td>
<td>38</td>
<td>0.546</td>
</tr>
<tr>
<td>Porphyra-334 = 21.57 + 0.0107(UVR4)</td>
<td>0.064</td>
<td>2.53</td>
<td>38</td>
<td>0.120</td>
</tr>
<tr>
<td>Porphyra-334 = 18.70 + 0.0234(UVR5)</td>
<td>0.072</td>
<td>2.87</td>
<td>38</td>
<td>0.099</td>
</tr>
<tr>
<td>Palythine = 1790 + 0.128(UVR4)</td>
<td>0.029</td>
<td>1.09</td>
<td>38</td>
<td>0.304</td>
</tr>
<tr>
<td>Palythene = 102.2 + 0.0470(UVR1)</td>
<td>0.087</td>
<td>3.83</td>
<td>41</td>
<td>0.057</td>
</tr>
<tr>
<td>Palythene = 103.7 + 0.0118(UVR4)</td>
<td>0.073</td>
<td>2.90</td>
<td>38</td>
<td>0.097</td>
</tr>
<tr>
<td>Palythene = 99.81 + 0.0264(UVR5)</td>
<td>0.085</td>
<td>3.42</td>
<td>38</td>
<td>0.072</td>
</tr>
</tbody>
</table>
Fig 2.5. *Porites compressa*. Mean tissue concentration of mycosporine-like amino acids (MAAs, nmol) normalized to surface area (cm$^{-2}$) at each of 14 collection dates from January 1998 to March 1999 (n = 3). Three MAAs were identified, (A) mycosporine-glycine, (B) asterina-330 and (C) shinorine. Error bars represent one standard error.
Fig 2.6. *Montipora verrucosa*. Mean tissue concentration of mycosporine-like amino acids (MAAs, nmol) normalized to dry skeletal mass (g) at each of 14 collection dates from January 1998 to March 1999 (n=3). Five MAAs were identified, (A) mycosporine-glycine, (B) palythine, (C) shinorine, (D) porphyra-334, and (E) palythene. Error bars represent one standard error.
Fig 2.7. Total mean tissue concentration of mycosporine-like amino acids (MAAs) in (A) *Porites compressa* and (B) *Montipora verrucosa* at each of 14 collection dates from January 1998 to March 1999 (n=3).
A. *Porites compressa*

B. *Montipora verrucosa*
**Photosynthetic pigments**

Four main photosynthetic pigments were found in the tissues of *P. compressa* and *M. verrucosa*: the porphyrins chl *a* and chl *c₂*, and the carotenoids, peridinin and diadinoxanthin (Fig. 2.8). Trace amounts of diatoxanthin and β-carotene were observed on the chromatograms (tentatively identified by retention times), but were not quantified.

Regression analysis revealed a significant inverse relationship between the number of days until or since the closest winter solstice (December 21) and the concentration of all four photosynthetic pigments of both species (Table 2.3) (Figs. 2.9 to 2.11). Number of days from winter solstice accounted for approximately 25% of the variation in total photosynthetic pigment concentration in both species. The regression models for diadinoxanthin showed a weaker relationship than the other pigments in *P. compressa* (*r² = 0.171*) and *M. verrucosa* (*r² = 0.188*).

In *M. verrucosa*, the only pigment-to-pigment ratio that displayed a pattern, and thus warranted regression analysis, was chl *c₂*:a*. There was a significant negative trend in value of the ratio with days from the closest winter solstice (*r² = 0.197*, *p<0.0032*). The ratio of the other accessory pigment, peridinin, to chl *a* did not change notably over the course of the year. Diadinoxanthin:chl *a* and diadinoxanthin to total pigments ratios also remained relatively constant throughout the year.

In *P. compressa*, neither of the accessory pigments, chl *c₂* or peridinin, changed in proportion to chl *a*. However, there was a clear positive relationship between diadinoxanthin:chl *a* and diadinoxanthin:total pigments and the number of days since the solstice (*r² = 0.158*, *p<0.0092* and *r² = 0.331*, *p<0.0001*, respectively).
Fig. 2.8. *Porites compressa*. Representative HPLC chromatogram of photosynthetic pigments from 100% methanolic extract. Chromatograms from *Montipora verrucosa* were of a like nature (not shown).
Table 2.3. Results of regression analysis for photosynthetic pigments in the tissues of *Porites compressa* and *Montipora verrucosa* and the number of days since or until the nearest winter solstice (December 21). P-values less than 0.1 are highlighted in bold.

<table>
<thead>
<tr>
<th>Model</th>
<th>$R^2$</th>
<th>F</th>
<th>df</th>
<th>P &lt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Porites compressa</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total pigments = 43034 - 92.4(days)</td>
<td>0.248</td>
<td>13.16</td>
<td>41</td>
<td>0.0008</td>
</tr>
<tr>
<td>Chlorophyll a = 16340 - 36.4(days)</td>
<td>0.224</td>
<td>11.51</td>
<td>41</td>
<td>0.0016</td>
</tr>
<tr>
<td>Chlorophyll a2 = 7365.5 - 17.87(days)</td>
<td>0.263</td>
<td>14.25</td>
<td>41</td>
<td>0.0005</td>
</tr>
<tr>
<td>Peridinin = 14816 - 30.7(days)</td>
<td>0.246</td>
<td>13.08</td>
<td>41</td>
<td>0.0008</td>
</tr>
<tr>
<td>Diadinoxanthin = 4512.5 - 7.44(days)</td>
<td>0.171</td>
<td>8.23</td>
<td>41</td>
<td>0.0065</td>
</tr>
<tr>
<td>Diadinoxanthin:Chl a = 0.273 + 0.000352(days)</td>
<td>0.158</td>
<td>7.49</td>
<td>41</td>
<td>0.0092</td>
</tr>
<tr>
<td>Diadinoxanthin:total pigments = 0.104 + 0.0000962(days)</td>
<td>0.331</td>
<td>19.81</td>
<td>41</td>
<td>0.0001</td>
</tr>
<tr>
<td><strong>Montipora verrucosa</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total pigments = 234464 - 512.4(days)</td>
<td>0.249</td>
<td>13.28</td>
<td>41</td>
<td>0.0008</td>
</tr>
<tr>
<td>Chlorophyll a = 89525 - 173.6(days)</td>
<td>0.181</td>
<td>8.81</td>
<td>41</td>
<td>0.0050</td>
</tr>
<tr>
<td>Chlorophyll a2 = 30830 - 80.9(days)</td>
<td>0.288</td>
<td>16.21</td>
<td>41</td>
<td>0.0002</td>
</tr>
<tr>
<td>Peridinin = 87094 - 201.4(days)</td>
<td>0.290</td>
<td>16.36</td>
<td>41</td>
<td>0.0002</td>
</tr>
<tr>
<td>Diadinoxanthin = 27015 - 56.6(days)</td>
<td>0.188</td>
<td>9.25</td>
<td>41</td>
<td>0.0041</td>
</tr>
<tr>
<td>Chl a2:Chl a = 0.348 - 0.000338(days)</td>
<td>0.197</td>
<td>9.81</td>
<td>41</td>
<td>0.0032</td>
</tr>
</tbody>
</table>
Fig 2.9. *Porites compressa*. Mean tissue concentration of photosynthetic pigments (µg) normalized to surface area (cm\(^{-2}\)) at each of 14 collection dates from January 1998 to March 1999 (n = 3). Four pigments were identified, (A) chlorophyll \(a\), (B) peridinin, (C) diadinoxanthin, and (D) chlorophyll \(c_2\). Error bars represent one standard error.
Fig 2.10. *Montipora verrucosa*. Mean tissue concentration of photosynthetic pigments (μg) normalized to dry skeletal mass (g) at each of 14 collection dates from January 1998 to March 1999 (n = 3). Four pigments were identified, (A) chlorophyll a, (B) Peridin, (C) diadinoxanthin, and (D) chlorophyll c₂. Error bars represent one standard error.
Fig. 2.11. Total mean tissue concentration of photosynthetic pigments in (A) *Porites compressa* and (B) *Montipora verrucosa* at each of 14 collection dates from January 1998 to March 1999 (*n* = 3).
A. *Porites compressa*

![Chart showing pigment levels for *Porites compressa*](chart1.png)

- Chlorophyll a
- Peridinin
- Diadinoxanthin
- Chlorophyll c₂

B. *Montipora verrucosa*

![Chart showing pigment levels for *Montipora verrucosa*](chart2.png)

- Chlorophyll a
- Peridinin
- Diadinoxanthin
- Chlorophyll c₂

Month: January (J), February (F), March (M), April (A), May (M), June (J), July (J), August (A), September (S), October (O), November (N), December (D), January (J), February (F), March (M)
DISCUSSION

In this study, concentrations of photosynthetic and some photoprotective molecules in the tissues of *P. compressa* and *M. verrucosa* correlated with factors relating to season. Total photosynthetic pigment concentration decreased by approximately 25% during the summer months of May, April and June compared to the winter months of December, January and February. MAAs, however, were surprisingly unresponsive to UVR levels measured during the three weeks prior to collection. Only two MAAs, shinorine in *P. compressa*, and palythene in *M. verrucosa*, displayed positive, although weak, relationships with UVR. It is possible that these species of coral maintain threshold levels of MAAs throughout the year.

*Mycosporine-like amino acids*

When compared to the well-documented changes in MAA concentration along a depth gradient (Dunlap et al. 1986; Banaszak et al. 1998), the annual changes observed in this study seem surprisingly small. When the accompanying changes in solar irradiance over these temporal/spatial scales are compared, however, the results become more understandable. Even in clear, oceanic water, irradiance is attenuated much more dramatically with depth than with season at tropical latitudes (Kirk 1994). As shown in this study, the amount of UVR measured at maximum solar altitude on a cloudless, winter day was 66% of that on a summer day. For an attenuation coefficient typical of coastal reef waters (*K*$_{UVR}$ = 0.315), a 66% change in UVR occurs by a depth of only 1.2 meters. Studies documenting depth gradients in MAA concentration have typically
ranged from 0 to 30 m wherein the light regime changes by orders of magnitude (Banaszak et al. 1998).

A study by Drollet et al. (1997) in Tahiti documented a significant, although weak ($p<0.05, r^2 = 0.31$) correlation between in vivo absorbance (300 to 360 nm) of mucus from eleven Fungia repanda colonies and UVR over an 80 week time interval. They found an even tighter correlation between the wavelength of maximum absorbance and UVR ($p<0.05, r^2 = 0.42$), suggesting that some MAAs are more responsive to changes in the UVR environment than other MAAs. Results presented here support this notion, providing evidence that shinorine is the most responsive in *P. compressa*, and palythene in *M. verrucosa*.

A study on the annual cycle of MAAs in the marine sponge, *Dysidea herbacea*, on the Great Barrier Reef also found somewhat equivocal results (Bandaranayake, 1997). Their data reveal, however, that mycosporine-glycine concentrations are higher during the summer. These authors suggested that some MAAs might perform roles other than UV photoprotection such as in reproductive and/or antioxidant processes. In the present study, mycosporine-glycine concentrations decreased in the summer months of June, July and August in *P. compressa*. This MAA is structurally different than the other MAAs found in this study in that it has a cyclohexenone ring as opposed to a cyclohexenimine ring (an oxygen atom instead of a nitrogen atom conjugated to the ring). Dunlap and Yamamoto (1995) demonstrated that mycosporine-glycine purified from the zooanthid, *Palythoa tuberculosa*, exhibits moderate antioxidant activity on a concentration-dependent basis. These authors suggest that mycosporine-glycine could potentially provide protection against photooxidative stress in the hyperoxic tissues of marine algal-
invertebrate symbioses. The observed decrease in mycosporine-glycine during the summer months of this study could reflect increased photooxidative stress associated with high irradiance levels resulting in the oxidation of mycosporine-glycine.

*Photosynthetic pigments*

Photoacclimation by *P. compressa* and *M. verrucosa* to seasonal changes in light availability was suggested in this study. Pigment levels on a surface area basis increased by a factor of 1.5 in winter months compared to summer months. In *M. verrucosa*, the ratio chl c2:chl a significantly decreased with the number of days from the winter solstice, suggesting photoacclimation by the zooxanthellae. The research of Iglesias-Prieto and Trench (1997) has shown that the light-harvesting apparatus of symbiotic dinoflagellates includes peripheral antennae composed of water-soluble peridinin-chlorophyll a-protein (PCP) complexes and membrane-bound antennae of chl a-chl c2-peridinin-protein (acpPC) complexes. Given this information, it would appear that in *M. verrucosa*, it is the chl c2-containing antennae that are being increased in size or number during the winter months.

In *P. compressa*, the proportion of diadinoxanthin making up the total pigment pool increased during the summer months. An increase in the total xanthophyll pool has been shown to occur in the coral *Goniastrea aspera* during a bleaching event in Thailand (Ambarsari et al. 1997). Also, cultured zooxanthellae from tropical symbioses become enriched in xanthophylls under supersaturating light conditions (Iglesias-Prieto and Trench 1997). In a free-living dinoflagellate, diadinoxanthin is converted to diatoxanthin during periods of high light and is thought to dissipate excess light energy via
fluorescence quenching (Demers et al. 1991). The existence of a xanthophyll cycle in coral-algal symbioses has just recently been confirmed for *Goniastrea aspera* and *Fungia fungites* in Phuket, Thailand (Brown et al. 1999). Light-mediated cycling between diadinoxanthin and diatoxanthin may have occurred during the period of coral collection in my study. Coral samples were always taken during daylight hours, but they were not flash-frozen. It is probable that any diatoxanthin present at the time of collection was converted back to diadinoxanthin as tissues froze in the dark. Regardless, the presence of diadinoxanthin and its proportional increase to the total pigment pool during the summer months revealed in this study supports a hypothesized photoprotective function of this xanthophyll in *P. compressa*.

The absence of a change in diadinoxanthin:chl a in *M. verrucosa* in this study is especially interesting in light of research by Iglesias-Prieto and Trench (1997). One strain of zooxanthellae investigated in their study was isolated from *M. verrucosa*. Unlike the two symbionts isolated from the jellyfish, *Cassiopeia xamachana*, and the zooanthid, *Zoanthus sociatus*, the symbiont from *M. verrucosa* did not show acpPC spectral evidence indicative of being enriched with a xanthophyll in the cultures grown in supersaturating light levels. They concluded that the three symbioses exemplify different levels of photoacclimative plasticity, each appropriate to their distribution in nature, with *M. verrucosa* being the most “shade-loving” species of the three examined. In the present study, *P. compressa* showed evidence of pigment pool enrichment by a xanthophyll during high-irradiance months, which is in accordance with its shallow (<15 m) distribution in Kaneohe Bay (pers. observation), while *M. verrucosa* did not show these trends.
Stimson (1997) documented an annual cycle in zooxanthella density within the tissues of *Pocillopora damicornis* in Hawaii. Mean density of zooxanthellae approximately doubled from summer to winter. Recently, Fagoonee et al. (1999) recorded the same phenomenon with six years of data for the coral, *Acropora formosa* in Mauritius; the densities of algal cells in autumn/winter were three times those in summer/spring. Since zooxanthellae densities were not measured in the present study, this form of photoacclimation cannot be confirmed for these two species of coral, but higher densities of zooxanthellae could have been partially responsible for the surface area specific increases in pigment concentration. However, the fact that the pigments changed in proportion to one another is evidence that zooxanthellae were photoacclimating by increasing the size and/or the number of light-harvesting antennae as light availability decreased.

**Conclusions**

This study demonstrates that the coral-algal symbioses of *M. verrucosa* and *P. compressa* adjust photosynthetic and photoprotective capacity on a seasonal cycle. Both symbioses increased surface area specific concentrations of photosynthetic pigments by a factor of 1.5 during the winter months. *M. verrucosa* also increased the proportion of chl c2:chl a during the winter. *M. verrucosa* was not very responsive to changes in the UVR environment; with the possible exception of palythene, MAA concentrations did not increase during summer. These results are in line with observations that *M. verrucosa* has its distribution maximum at depths >2 m in Kaneohe Bay. In *P. compressa*, concentrations of the MAA shinorine and the proportion of diadinoxanthin in the total
pigment pool increased during spring/summer months. Both of these adjustments are in line with expectations for survival in high-irradiance, high-UVR environments.
CHAPTER 3: THE EFFECTS OF ULTRAVIOLET RADIATION AND WATER MOTION ON THE REEF CORAL, *Porites compressa* Dana. I. FLUME

INTRODUCTION

Ultraviolet radiation (UVR, 280 to 400 nm) is an important environmental factor that has greatly influenced the evolution of life. In tropical regions, the flux of UVR is at its global extreme, and the coastal waters are characteristically clear and nutrient poor (Holm-Hansen et al. 1993; Kirk 1994). Oligotrophic waters are highly transparent to UVR, and UVR penetration is common to depths of 30 m (Jerlov 1950; Smith and Baker 1979). Organisms that have adapted to low nutrient, high irradiance environments include hermatypic scleractinians. The dinoflagellate symbionts (zooxanthellae) living within the gastrodermis of the coral are autotrophic, and contribute substantially to the carbon budget of the symbiosis (Muscatine 1990). The ecological distribution of the symbiosis suggests the evolutionary development of UVR protective mechanisms, for it is well established that nucleic acids and other cellular constituents are damaged by UVR (Quaite et al. 1992; Holm-Hansen et al. 1993).

A family of UV-absorbing compounds was first extracted from coral tissue by Shibata (1969) and termed the ‘S 320’ complex because of the compounds’ absorbance properties from 310 to 380 nm. These compounds are now referred to as mycosporine-like amino acids (MAAs) due to their structural similarity to the mycosporines found in sporulating fungi (Leach 1965; Favre-Bonvin et al. 1976). MAAs have been isolated from the tissues of marine organisms across many phyla of the animal kingdom (Dunlap
and Chalker 1986; Karentz et al. 1991), as well as phytoplankton (Carreto et al. 1990; Lesser 1996), macroalgae (Beach et al. 1997; Banaszak et al. 1998), and cyanobacteria (Garcia-Pichel and Castenholz 1993). There are presently 19 known MAAs, all sharing the same basic molecular structure of a cyclohexenone or cyclohexenimine ring conjugated with the nitrogenous component of an amino acid (Dunlap and Shick 1998).

The single known biosynthetic source for MAAs is the shikimate-acid pathway, found only in photosynthetic bacteria, marine algae and fungi (Bentley 1990). Corals may acquire MAAs from the zooxanthellae, from diet, or both (Dunlap and Shick 1998).

Given the ubiquity of MAAs throughout the marine ecosystem and their absorbance properties, a photoprotective role was postulated (Carreto et al. 1990; Karentz et al. 1991; Garcia-Pichel et al. 1993). The discovery that MAA concentration in corals is inversely correlated with depth is strong circumstantial evidence of the photoprotective role of MAAs (Dunlap et al. 1986). A study by Shick et al. (1995) demonstrated the inducible nature of the compounds by transplantation along a depth gradient on the Great Barrier Reef. *Acropora microphthalma* colonies transplanted to depths of 2 and 10 m acquired five to ten-fold higher concentrations of MAAs than those relocated to 20 and 30 m depth. In Hawaii, a survey conducted comparing corals from two sites showed that MAAs dropped to negligible concentrations at depths at which UVR was still detected in corals at an offshore reef, but not in those collected at a murky estuarine site nearby (Banaszak et al. 1998). These results suggest that additional factors related to depth may influence MAA concentration besides UVR.

UVR attenuates exponentially with water column depth (Jerlov 1950; Fleischmann 1989), as do photosynthetically active radiation (PAR) (Kirk 1994) and
water motion (Helmuth et al. 1997b). Water motion is positively correlated to photosynthetic rate in coral (Dennison and Barnes 1988; Patterson et al. 1991; Lesser et al. 1994), coral growth rate (Jokiel 1978), and phosphate uptake rate by coral reefs (Atkinson and Bilger 1992). There is general agreement that these phenomena result from the reduction in the thickness of the diffusion boundary layer (DBL), allowing higher exchange rates of gaseous substrates and waste products utilized in photosynthesis and other metabolic activities. If MAA production and/or accumulation are limited by the uptake rate of any substrate, MAA concentration may be affected by water motion. The factors controlling the synthesis and accumulation of MAAs in the coral-algal symbiosis (CAS) have not been determined to date.

The aim of this study was to test the effects of water motion and UVR on P. compressa in a factorial experiment. The parameters measured include MAA concentration, chlorophyll a (chl a) concentration, and calcification rate. It was hypothesized that the rate of MAA accumulation may be affected by water motion, but in time, the concentration of MAAs in the two flow regimes would converge at a level determined by the dose of UVR. Thus, an interaction between water flow and time is expected. In the absence of UVR, corals are expected to lose their MAAs regardless of water motion regime. Studies have shown that zooxanthellae are nitrogen-limited in hospite (Hoegh-Guldberg and Smith 1989; Stambler et al. 1991; Marubini and Davies 1996; Muscatine et al. 1998). MAAs contain one to two nitrogen atoms per molecule (Teai et al. 1998), and thus may be expensive compounds to produce under nitrogen limitation. Calcification rate and chl a concentration were measured during the experiment in order to estimate the metabolic burden of UVR and/or production of
MAAs to the CAS. If ambient levels of UVR procure a cost to the CAS, removing the UVR from the environment should result in an increase in skeletogenesis and/or photosynthetic pigment concentration.
MATERIALS AND METHODS

Experimental manipulations

Branches of *Porites compressa* Dana were harvested from the windward reef flat of Coconut Island in Kaneohe Bay, Hawaii on June 27, 1995. To eliminate genetic variability as a source of within treatment variance, all coral samples were taken from a single male colony at 2 m depth. PAR measurements using a LiCor® LI-188B integrating quantum photometer revealed that approximately 45% of surface irradiance reaches that depth. Individual nubbins were taken from one area of the colony and transported to the lab for preparation. Each coral nubbin was filed smooth at the break-off point, and attached with Super Glue™ to individual 2.5 x 2.5 cm ceramic tiles (Davies 1995). All coral nubbins were allowed thirteen days to heal areas of exposed skeleton and to acclimate to the water table conditions. During this time, moderate water motion was created with an air stone and light levels were manipulated with shade cloth to ≈ 45% of surface irradiance. Within one week of this healing period, the corals had begun to extend living tissue down onto the tile. At the end of the healing period corals were numbered, labeled, and then randomly assigned to four treatments. The treatments included UV-opaque high flow (UVO HI), UV-transparent high flow (UVT HI), UV-opaque low flow (UVO LO), and UV-transparent low flow (UVT LO). Coral nubbins remained in their respective treatments for a total of six weeks (July 10 – August 22, 1995).

The experiment took place in an outdoor flow-through flume. The flume was constructed of a 15.3 cm diameter, 3.4 m long PVC pipe cut in half longitudinally and
placed in a 3.6 m long x 35 cm wide x 32 cm deep rectangular sea table. The half-pipes were suspended side by side in the tank such that the edges of the pipes rose above the water line, resulting in two flumes, 11 cm deep. To create the two water motion treatments, a large air stone was placed at the end of the high-flow pipe forcing the water down the pipe and into a reservoir area that gently supplied the low-flow pipe (i.e., all treatments were in the same body of water). The tank in which the flumes were suspended was continuously supplied with seawater from the reef at a change over rate of \( \approx 12 \) l min\(^{-1}\). Dissolved oxygen measurements were taken once during the experiment in all regions of each flume. The water motion was measured daily by timing a neutrally buoyant polyethylene sphere as it traveled through each flume. Water temperature measurements were taken daily.

Placing a sheet of UV-transparent (UVT) Plexiglas over one end of the flume, and a UV-opaque (UVO) sheet over the other half imposed the UVR treatments. Each sheet of plastic was scanned in a spectrophotometer before and after the experiment to check for weathering. Neutral density mesh was placed equally over all treatments, approximating irradiance levels found at 2 m depth in the field. Coral nubbin positions were rotated within treatments every three days to limit positional effects within the flumes. A dummy coral nubbin was placed at the start of each row so that no experimental nubbin was exposed to the undisturbed flow at the head of the flume. The location of the treatments within the flume were also rotated weekly to minimize positional effects. All corals were weighed initially (July 9) and after 3 weeks (August 3) and 6 weeks (August 22). Nubbins \((n = 9)\) were also harvested at those times for determination of MAA and chl a concentration.
**HPLC analysis of mycosporine-like amino acids**

Coral nubbins were extracted in 10 ml of 100% HPLC grade methanol for exactly 24 hours at -20°C. The analysis of MAAs was performed by slightly modifying procedures described in Dunlap and Chalker, (1986). Using a Shimadzu® system, 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 of 0.1% acetic acid and 25% methanol. Detection of peaks was by UVR absorbance at 313 and 340 nm, and flow rate was 0.5 ml min⁻¹. Identities of peaks were confirmed by co-chromatography with quantitative standards of mycosporine-glycine, shinorine, porphyra-334, palythine, asterina-330, palythinol, and palythene (graciously provided by W.C. Dunlap, Australia Institute of Marine Science). Quantification of individual MAAs was accomplished by calibrating the system using the quantitative standards, and peaks were integrated using EZChrom™ software (Shimadzu® Inc.). MAA concentrations were normalized to nubbin surface area, which was determined using the aluminum foil technique (Marsh 1970).

**Measurement of chl a concentration**

Chl a concentration was determined spectrophotometrically by applying a standard curve prepared using pure chl a (Sigma Chemical Company). Absorbance was measured at 666 nm and at 750 nm, and the latter subtracted from the first to adjust for
suspended particulate matter. These measurements were made on the same MeOH extracts used for the HPLC analysis.

Measurement of calcification rate

Skeletogenesis was quantified by measuring the increase in calcium carbonate skeleton using the buoyant weight technique described by Jokiel et al. (1978). Increase in dry weight was then cube-root transformed into a linear dimension of growth in order that the measurement is independent of the coral's original size. This transformation was accomplished using the formula for "mean solid radius" from Maragos (1972). The utility of this transformation is discussed in Buddemeier and Kinzie (1976).

\[ R = 3\sqrt[3]{\frac{3W}{2\pi D}} \]

Where \( R \) = mean solid radius
\( W \) = net dry weight of aragonite gained per day
\( D \) = skeletal density of *Porites compressa* (1.41 g/cm\(^3\))

Statistical analysis

Each coral nubbin was measured only once during the experiment. The effects of UVR, water motion (flow) and time on the response variables MAA concentration, chl \( a \) concentration and calcification rate, were analyzed using three-way Model I (fixed factor) ANOVA. A two-way Model I ANOVA was also performed on the six-week data for calcification rate. All models contained each two-way and the three-way interaction term. The assumptions of equal variance and the aptness of the models were examined by considering residual plots including residuals vs. predicted values, residuals vs. the order in which the samples were run, and normal probability plots. In determining the
aptness of the three-way ANOVA for the MAA concentration data, the residuals were plotted versus the order in which the samples were run on the HPLC. A regression analysis of this plot resulted in a significant model ($p<0.0019$), probably signifying the degradation of the performance of the HPLC column as it was loaded with samples. Because there was a linear relationship between the residuals and the order in which the samples were run, the slope of the regression line was used as an adjustment. The equation used was $0.3317(\text{order}) + \text{original MAA concentration} = \text{corrected MAA concentration}$. This transformation did not change any of the conclusions of the analysis.
RESULTS

Experimental conditions

During the course of the study, nubbins added polyps onto the ceramic tiles upon which they were mounted, in some cases covering the entire top surface by the end of six weeks. Polyp behavior was typical of this species, extended for feeding both night and day, with occasional production of mucus sheets (Croffroth 1991) in a small percentage (≈ 10%) of the colonies. The mean water motion in the high and low flow flumes was $14.6 \pm 1.4 \text{ cm s}^{-1}$ ($n=29$) and $3.1 \pm 0.6 \text{ cm s}^{-1}$ ($n=29$), respectively. The flow was turbulent and had a Reynolds number of approximately $2.95 \times 10^4$ in the high flow flume and $5.66 \times 10^3$ in the low flow flume, assuming fully developed flow (M. Baird, pers. comm.). Water motion was not measured at the original site of coral collection, but measurements made by Lesser et al. (1994) reported values ranging from $3.3 \pm 2.5$ to $7.8 \pm 4.5 \text{ cm sec}^{-1}$ for similar habitats near the collection site. Dissolved oxygen measurements revealed that the water was supersaturated in both of the flumes. Daily temperature measurements averaged $27.3 \pm 0.4^\circ \text{C}$ (mean ± SD, $n=30$).

Mycosporine-like amino acid concentration

The presence of UVR had a significant and positive effect on the concentration of MAAs in *Porites compressa* ($p<0.0001$) (Table 3.1) (Fig. 3.1). There was, however, a significant three-way interaction between UVR, water motion and time ($p<0.0054$), indicating that time and water motion were having indirect effects on MAA concentration. In the absence of UVR, total MAA concentrations decreased continually
throughout the experiment. In the presence of UVR, total MAA concentrations increased
during the first three weeks in both water motion treatments, but at a much faster rate in
high water motion. During the second three weeks total MAA concentrations declined in
the UVT HI treatment, but continued to increase in the UVT LO treatment, evidenced by
a significant water motion x time interaction (p<0.0469). Thus, at the end of six weeks,
corals in the UVT LO treatment had the highest mean concentration of MAAs, followed
by the UVT HI, the UVO HI and the UVO LO treatment.

Three individual MAAs were identified in the methanol extractions: asterina-330,
shinorine, and mycosporine-glycine. There was a small fourth peak evident in the
chromatograms from detection at 313 nm, the identity of which could not be determined,
but the area of this peak was always less than 10% of the total peak area at 313 nm, and
barely detectable at 340 nm. Asterina-330 was the most abundant MAA in the samples,
ranging from 40 to 100 nmol cm\(^{-2}\), followed by shinorine from 8 to 17 nmol cm\(^{-2}\), and
mycosporine-glycine from one to five nmol cm\(^{-2}\). Asterina-330 displayed a similar
pattern to the total MAA results discussed above, as it was the main component of the
MAA pool (Fig. 3.1) (Table 3.1). The UVR effect was significant (p<0.0001). The
three-way interaction (p<0.0043) and the two-way water motion x time interaction
(p<0.0493) were also significant. Asterina concentrations only decreased by 7.5% in
corals in the UVO HI treatment, but by 25.6% in the UVO LO treatment. For
mycosporine-glycine, the assumption of equal variance within treatments was not met
(the data did not pass the Hartley Test for equality of variances at alpha = 0.01) (Neter et
al. 1990). To remedy this, the data were log\(_{10}\) transformed. This transformation solved
the unequal variance problem, and did not change any of the conclusions drawn from the
ANOVA. The UVR effect was significant (p<0.0001), along with the three-way interaction (p<0.0044). Mycosporine-glycine concentrations decreased at a rate of 0.510 nmol cm^{-2} week^{-1} during the first three weeks in corals shaded from UVR (resulting in a 40 % loss) and then leveled off, changing very little during the latter three weeks of the experiment. Shinorine exhibited a much different response to the treatments than the other MAAs in that it decreased in concentration in all treatments during both time periods (time p<0.0001) (Fig.3.1) (Table 3.1). However, Shinorine still displayed a positive response to the presence of UVR (p<0.0001) although there was a significant interaction between UVR and time (p<0.0037), apparent as the development of the UVR effect during the second half of the experiment. The fourth, unidentified compound did not respond to UVR or water motion, but decreased in mean peak area throughout the experiment at an overall rate of approximately 295 a.u. week^{-1} (time p<0.0001).
Table 3.1. Results of three-way ANOVA for Total MAA concentration, mycosporine-glycine, asterina-330, shinorine, the unknown compound, chl a, and calcification rate. Results based upon the SAS Institute's general linear model procedure. MS = mean square. *F*-values less than 0.05 highlighted in bold.

<table>
<thead>
<tr>
<th>Dependent Variable</th>
<th>UVR</th>
<th>Flow</th>
<th>Time</th>
<th>UVR x Time</th>
<th>Flow x Time</th>
<th>UVR x Flow</th>
<th>Error</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total MAAs</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>df</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>64</td>
</tr>
<tr>
<td>MS</td>
<td>20926</td>
<td>372.9</td>
<td>741.7</td>
<td>19.27</td>
<td>438.0</td>
<td>1587</td>
<td>3203</td>
<td>386.6</td>
</tr>
<tr>
<td>F</td>
<td>54.13</td>
<td>0.96</td>
<td>1.92</td>
<td>0.05</td>
<td>1.13</td>
<td>4.11</td>
<td>8.28</td>
<td></td>
</tr>
<tr>
<td>p</td>
<td>&lt;0.0001</td>
<td>&gt;0.10</td>
<td>&gt;0.10</td>
<td>&gt;0.10</td>
<td>&gt;0.10</td>
<td>&lt;0.047</td>
<td>&lt;0.0054</td>
<td></td>
</tr>
<tr>
<td><strong>Mycosporine-glycine</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>df</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>64</td>
</tr>
<tr>
<td>MS</td>
<td>1.385</td>
<td>0.03860</td>
<td>0.001766</td>
<td>0.001756</td>
<td>0.01685</td>
<td>0.006794</td>
<td>0.09947</td>
<td>0.01142</td>
</tr>
<tr>
<td>F</td>
<td>121.3</td>
<td>3.38</td>
<td>0.15</td>
<td>0.15</td>
<td>1.48</td>
<td>0.60</td>
<td>8.71</td>
<td></td>
</tr>
<tr>
<td>p</td>
<td>&lt;0.0001</td>
<td>&lt;0.0706</td>
<td>&gt;0.10</td>
<td>&gt;0.10</td>
<td>&gt;0.10</td>
<td>&gt;0.10</td>
<td>&lt;0.049</td>
<td>&lt;0.0044</td>
</tr>
<tr>
<td><strong>Asterina-330</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>df</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>64</td>
</tr>
<tr>
<td>MS</td>
<td>16911</td>
<td>244.2</td>
<td>319.6</td>
<td>0.1715</td>
<td>405.6</td>
<td>1405</td>
<td>3068</td>
<td>349.7</td>
</tr>
<tr>
<td>F</td>
<td>48.35</td>
<td>0.70</td>
<td>0.91</td>
<td>0.00</td>
<td>1.16</td>
<td>4.02</td>
<td>8.77</td>
<td></td>
</tr>
<tr>
<td>p</td>
<td>&lt;0.0001</td>
<td>&gt;0.10</td>
<td>&gt;0.10</td>
<td>&gt;0.10</td>
<td>&gt;0.10</td>
<td>&gt;0.10</td>
<td>&lt;0.049</td>
<td>&lt;0.0043</td>
</tr>
<tr>
<td><strong>Shinorine</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>df</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>64</td>
</tr>
<tr>
<td>MS</td>
<td>35.88</td>
<td>7.088</td>
<td>83.23</td>
<td>16.85</td>
<td>0.2278</td>
<td>1.020</td>
<td>1.431</td>
<td>1.856</td>
</tr>
<tr>
<td>F</td>
<td>19.33</td>
<td>3.82</td>
<td>44.84</td>
<td>9.08</td>
<td>0.12</td>
<td>0.55</td>
<td>0.77</td>
<td></td>
</tr>
<tr>
<td>p</td>
<td>&lt;0.0001</td>
<td>&lt;0.0552</td>
<td>&lt;0.0001</td>
<td>&lt;0.0037</td>
<td>&gt;0.10</td>
<td>&gt;0.10</td>
<td>&gt;0.10</td>
<td></td>
</tr>
<tr>
<td><strong>Unknown compound</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>df</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>64</td>
</tr>
<tr>
<td>MS</td>
<td>3519</td>
<td>938770</td>
<td>12847651</td>
<td>37651</td>
<td>16342</td>
<td>792681</td>
<td>466131</td>
<td>339333</td>
</tr>
<tr>
<td>F</td>
<td>0.01</td>
<td>2.77</td>
<td>37.86</td>
<td>0.11</td>
<td>0.05</td>
<td>2.34</td>
<td>1.37</td>
<td></td>
</tr>
<tr>
<td>p</td>
<td>&gt;0.10</td>
<td>&gt;0.10</td>
<td>&lt;0.0001</td>
<td>&gt;0.10</td>
<td>&gt;0.10</td>
<td>&gt;0.10</td>
<td>&gt;0.10</td>
<td></td>
</tr>
<tr>
<td><strong>Chlorophyll a</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>df</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>64</td>
</tr>
<tr>
<td>MS</td>
<td>1.446</td>
<td>18.71</td>
<td>6.716</td>
<td>0.01531</td>
<td>1.008</td>
<td>0.7892</td>
<td>0.1496</td>
<td>0.5745</td>
</tr>
<tr>
<td>F</td>
<td>2.52</td>
<td>32.57</td>
<td>11.69</td>
<td>0.03</td>
<td>1.75</td>
<td>1.37</td>
<td>0.26</td>
<td></td>
</tr>
<tr>
<td>p</td>
<td>&gt;0.10</td>
<td>&lt;0.0001</td>
<td>&lt;0.0011</td>
<td>&gt;0.10</td>
<td>&gt;0.10</td>
<td>&gt;0.10</td>
<td>&gt;0.10</td>
<td></td>
</tr>
<tr>
<td><strong>Calcification rate</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>df</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>80</td>
</tr>
<tr>
<td>MS</td>
<td>0.001090</td>
<td>0.01210</td>
<td>0.09952</td>
<td>0.005352</td>
<td>0.0005180</td>
<td>0.0008500</td>
<td>0.001776</td>
<td>0.0007860</td>
</tr>
<tr>
<td>F</td>
<td>1.39</td>
<td>15.40</td>
<td>126.62</td>
<td>6.81</td>
<td>0.66</td>
<td>1.08</td>
<td>2.26</td>
<td></td>
</tr>
<tr>
<td>p</td>
<td>&gt;0.10</td>
<td>&lt;0.0002</td>
<td>&lt;0.0001</td>
<td>&lt;0.0108</td>
<td>&gt;0.10</td>
<td>&gt;0.10</td>
<td>&gt;0.10</td>
<td></td>
</tr>
</tbody>
</table>

* ANOVA performed on log transformed data due to unequal variance among treatments.
Fig. 3.1. Mean concentration of mycosporine-like amino acids in corals normalized to surface area ± standard error (n = 9) versus time. UVT = ambient UV, UVO = no UV, HI = high water motion, LO = low water motion.
Chlorophyll $a$ concentration

A gradual darkening of coral nubbins in both of the high water flow treatments was observed over the course of the experiment. Mean chl $a$ concentration was significantly enhanced by water motion ($p<0.0001$) and time ($p<0.0011$) (Table 3.1) (Fig. 3.2). Nubbins placed in high water motion increased in chl $a$ concentration by an average of 33% by the end of six weeks. Mean chl $a$ concentration in the low water motion corals declined during the first three weeks of the experiment, but returned to ambient levels by the end of six weeks.

Calcification rate

Calcification rates increased in all treatments from the first to the second half of the experiment (Time $p<0.0001$) (Table 3.1) (Fig. 3.3). Calcification was slower in high water motion than in low water motion during the course of the experiment (flow $p<0.0002$), particularly during the first three weeks. There was a significant interaction between UVR and time ($p<0.0108$), indicating that a UVR effect developed during the second three weeks. Calcification rate was significantly slower in the UVT HI (high water motion with UVR present) treatment than in the other three treatments during this time period (Tukey-adjusted multiple comparison of means using six-week data ($p<0.0263$)).
Fig. 3.2. Mean chlorophyll a concentration in coral nubbins normalized to surface area ± standard error (n = 9) versus time. UVT = ambient UV, UVO = no UV, HI = high water motion, LO = low water motion.
Fig. 3.3. Calcification rate in mean solid radius ± standard error (n = 11) versus time. UVT = ambient UV, UVO = no UV, HI = high water motion, LO = low water motion.
DISCUSSION

Several studies have documented a correlation between MAAs and UVR (Gleason 1993; Shick et al. 1995; Banaszak et al. 1998). Here, we see evidence that *Porites compressa* responds to alterations of the UVR regime by adjusting the total concentration of MAAs within the colony. MAA concentration was enhanced in corals kept in the presence of UVR, and reduced in corals kept in the absence of UVR. High water motion enhanced the rate of MAA accumulation during the first three weeks: corals in high flow with UVR present increased MAAs at a rate nearly four-fold greater than the corals in low flow with UVR present. These results are consistent with the notion that MAA synthesis, for nubbins from this coral colony, is limited by mass transport of some substrate(s), which can be increased by thinning of the diffusion boundary layer via increasing water motion (Patterson et al. 1991; Atkinson and Bilger 1992). During the second three weeks of the experiment, however, the mean MAA concentration in the nubbins of the UVT HI treatment dropped below the UVT LO treatment, while MAA concentrations in the nubbins of the UVT LO treatment continued to increase. The chl a concentration and calcification rate results may help clarify this apparent paradox, for they are important physiological indicators of the symbiotic relationship.

Chl a concentration per colony surface area was enhanced by high water motion, suggesting that the zooxanthellae were either increasing in numbers, producing more photosynthetic pigment, or both. However, there was no UVR effect on the concentration of chl a in this study. Similarly, Jokiel and York (1982) found no UVR effect on chl a, chl c or density of zooxanthellae in *Pocillopora damicornis* in Hawaii. These results
indicate that ambient levels of UVR do not inhibit the production or functioning of photosynthetic pigments in at least two species of coral. The significant effect of time on chl a concentration suggests that nubbins in all treatments were acclimating to some factor in the experimental environment not accounted for. The mean solar irradiance and the mean water temperature during the two three-week periods were not significantly different from each other (two sample t-tests, p<0.31 (n = 21) and p<0.78 (n = 13), respectively), so it is unlikely that these environmental parameters drove the significant time effect.

All coral nubbins calcified approximately 14% faster during the second three weeks than the first three weeks of the experiment. During the first three weeks of the experiment, corals had only begun to lay new polyps onto the tiles. By the end of six weeks, however, most of the corals had new polyps almost completely covering the upper surface of the tiles. This lateral extension of the colonies could account for the acceleration of growth during the latter half of the experiment. According to Dustan (1975), two types of skeletal addition can be observed in corals: that along the upward axis as individual polyps accrete aragonite, and that along a horizontal axis as new polyps are added. Considering that a new axis of growth commenced during the second half of the experiment, it is not surprising that calcification rates accelerated. However, acclimation of the corals to the imposed treatments may also have had a positive effect on growth.

In this study, corals subjected to high water motion did not calcify as fast as corals in low water motion during the first three weeks. During the second three weeks, however, corals in high flow with UVR present grew slower than the rest. In contrast,
*Acropora formosa* colonies (Houtman Abrolhos, Western Australia) naturally occurring at a protected site have greater branch extension rates than corals at a wave-exposed site, although mass increase per branch does not differ between sites (Harriott 1998). Further, the skeletal growth rate of *Pocillopora damicornis* in Hawaii is enhanced by the removal of UVR (Jokiel and York 1982). I did not see such a release in growth rate by removing UVR in this experiment, but the combination of high water motion and UVR may have acted synergistically to keep growth rates lower compared to the other treatments during the second three weeks. It is possible that a flow rate of 14.6 cm s\(^{-1}\) may be near the upper limit of the range in water motion that *P. compressa* normally experiences, causing a low level of stress to the nubbins in the high water motion treatments. This species is mainly a low water motion coral, being replaced by *Porites lobata* in habitats characterized by high wave exposure (Devaney and Eldredge 1977).

The inconsistent response of coral nubbins in the UVT HI treatment warrants some discussion. The nubbins in this treatment had the highest mean chl a concentrations throughout the experiment. During the first three weeks, these nubbins accumulated MAAs at a rate nearly four times that of nubbins exposed to UVR in low water motion. During the second three weeks, however, MAA concentrations in the UVT HI nubbins fell to levels similar to that at time zero. As discussed above, these nubbins also grew slower than nubbins in the other treatments during this time period. One interpretation is that high chlorophyll levels (or high zooxanthella densities) caused self-shading within the coral polyps, allowing the symbiosis to decrease levels of MAAs without leaving tissues vulnerable to UVR. Conversely, perhaps the corals were declining in health as the zooxanthellae flourished due to the high water motion. Recent research has shown that
nitrification of the water in which corals are grown can enhance zooxanthella densities and decrease calcification rates in *Porites porites* and *Montastrea annularis* (Marubini and Davies 1996). Stambler et al. (1991) saw a similar slowed calcification rate in response to nitrification in *Pocillopora damicornis*, and suggested that the zooxanthellae were using the products of photosynthesis to augment their own growth at the expense of the coral. Muscatine (1998) recently introduced the idea of host cell carrying capacity, and reported that elevated ammonium levels resulted in a significant increase in the proportion of host cells containing two algal cells instead of one. The present study suggests that the effect of high water motion on calcification rate may be similar to that of adding nitrogen. Decreasing the thickness of the diffusive boundary layer, allowing increased rates of nutrient uptake, could have augmented zooxanthella populations. A slower acceleration of calcification rate in the UVT HI treatment during the second three weeks supports this argument.

Because of the fact that only a single, male colony of *P. compressa* was used in this study, generalizations regarding the strategies for UVR protection in this species should be made with caution. It is possible that female colonies or colonies of different genotypes accumulate and use MAAs in a different manner. Now that it is known that the effects discovered in this study exist for this genotype, they should be tested for in a broader setting including different genotypes and species.

In conclusion, this study revealed that exposure to UVR determined the MAA concentration found in the tissues of this colony of *P. compressa*. The inducible nature of the compounds by the presence of UVR supports the notion that MAAs are of a photoprotective nature. The MAA concentrations remained high in the presence of UVR,
decreased in the absence of UVR, and were not directly affected by water flow. The significant interactions among the factors tested indicate that the accumulation of MAAs is a complex process, indirectly affected by metabolic processes associated with water flow, the physiological state of the coral, and the activities of the zooxanthellae. The fact that the symbiosis does not sustain levels of MAAs when UVR is not present suggests that there may be a cost associated with maintaining the compounds.
INTRODUCTION

Ultraviolet radiation (UVR, 280 - 400) is an important environmental factor in marine ecosystems (Hader and Worrest 1991). There have been measured increases in UV-B (280-320 nm) radiation at high latitudes associated with decreases in stratospheric ozone concentration (Farman et al. 1985; Frederick and Snell 1988; Kerr and McElroy 1993). Since this discovery, research efforts have focused on determining the possible ramifications of the increase in UV-B for life in the ocean (Hader and Worrest 1991; Shick et al. 1996; Franklin and Forster 1997). It is particularly important to study pre-existing photoprotective mechanisms that organisms from high solar irradiance environments exhibit to mitigate the effects of ambient UVR.

Coral reef organisms are likely species to exhibit a diversity of UVR protective mechanisms. In tropical regions, the flux of UVR is extremely high, associated with the low zenith angle of the sun and naturally low concentrations of stratospheric ozone. The incident UVR under the austral spring ozone hole does not approach values regularly experienced by organisms at low latitudes during the summer solstice (Frederick and Snell 1988). The waters surrounding coral reefs are characteristically nutrient poor and highly transparent to UVR, which can penetrate to depths of 30 m (Jerlov 1950; Smith and Baker 1979). Translating this depth estimate to a biologically effective dose (1% damage), UVR can affect organisms at least 6 m deep, even at moderately productive reef
locations (Dunne and Brown 1996). Thus, UVR is an important parameter structuring coral reef communities; cryptic fauna can lack protective mechanisms to survive in ambient levels of UVR found in shallow reef habitats (Jokiel 1980). One of the most ubiquitous orders of animals that has adapted to shallow, high irradiance habitats is the scleractinian corals. The coral-algal symbiosis depends upon photosynthetically active radiation (PAR) for algal photosynthesis. Coral polyps extend beyond the calcium carbonate skeleton and are subjected to high levels of PAR and UVR. This arrangement suggests the evolution of protective mechanisms, for it is well established that DNA, RNA and proteins absorb and are damaged by ambient levels of UVR (Calkins and Thordardottir 1980; Quaite et al. 1992).

Coral tissues contain substances that absorb radiation in the 310 to 400 nm range (Shibata 1969). Now referred to as mycosporine-like amino acids (MAAs), these compounds have been identified in the tissues of many marine organisms including most benthic invertebrates, macroalgae, phytoplankton, and photosynthetic bacteria (see Dunlap and Shick 1998 for a review). Both the ubiquity of this family of compounds and their absorbance properties have led to the interpretation that MAAs have a photoprotective function (Carreto et al. 1990; Karentz et al. 1991; Garcia-Pichel et al. 1993). Many studies have shown that MAA concentration strongly correlates with UVR dose in corals collected from the field (Maragos 1972; Dunlap et al. 1986; Banaszak et al. 1998). Experimentally, concentrations of MAAs decrease when the UVR is removed for coral (Kinzie 1993) and macroalgae (Wood 1989). Reciprocally, MAA concentration can be enhanced by artificially increasing the dose of UVR for sea anemones and jellyfish (Banaszak and Trench 1995), terrestrial cyanobacteria (Garcia-Pichel et al. 1998).
1993), diatoms (Riegger and Robinson 1997), and dinoflagellates (Lesser 1996). In the field, corals transplanted from 6 m to 1 m depth were shown to increase MAA concentration in the presence of UVR, but not when it was screened out with filters (Gleason 1993). However, changes in MAA concentration are not completely determined by UVR regime; water motion and PAR may also play a role, at least for *Pocillopora damicornis* (Jokiel et al. 1997). They suggest that increases in photosynthetic rate associated with increases in water motion could result in concurrent increases in MAA concentration via reduction in diffusive boundary layer thickness with higher water motion.

This study examines how UVR and water motion may affect the concentration of MAAs in the tissues of the reef coral, *Porites compressa*. In addition, I measured calcification rate and concentrations of photosynthetic pigments to estimate the metabolic burden of UVR and/or maintaining high levels of MAAs to the coral-algal symbiosis. No study to date has examined possible synergistic effects of UVR and water motion on MAA concentration or any other physiological parameters for reef corals. Water motion is positively related to zooxanthellar rates of photosynthesis *in hospite* (Dennison and Barnes 1988; Patterson et al. 1991; Lesser et al. 1994; Rex et al. 1995), coral growth and reproduction rates (Jokiel 1978), as well as larger-scale phosphate uptake across reef flats (Atkinson and Bilger 1992). These phenomena have been explained by the reduction in the thickness of the diffusive boundary layer (DBL), allowing higher rates of delivery and removal of metabolic substrates and waste products. Thus, there is reason to believe that MAA concentration could be affected by water motion, resulting in a cascade of effects on the coral-algal symbiosis.
I hypothesize that in the absence of UVR, MAA concentration will decrease regardless of MAA concentration. In the presence of UVR, I expect MAA concentration to remain high, but possibly be lower in the coral nubbins placed at the low water motion site due to boundary layer effects. In addition, corals exposed to UVR will have lower concentrations of photosynthetic pigments. If there is a cost associated with the production of MAAs, then in the absence of UVR, zooxanthellae should have increased access to resources to apply towards growth and/or production of photosynthetic pigments, resulting in an increased rate of coral calcification.
MATERIALS AND METHODS

Preparation of coral replicates

Branches of *Porites compressa* were harvested from nine colonies on the windward reef of Coconut Island, Kaneohe Bay, Hawai‘i, on June 19, 1997. These nine colonies were selected on the basis of depth (1.5 m) and the presence of suitable finger-like extensions for easy removal of coral fragments. Fourteen "nubbins" (single, non-bifurcated branches) were taken from each colony by snipping with bone shears from the top of the colony. Upon return to the laboratory, bases of nubbins were ground smooth and attached with marine epoxy to individual 10 x 10 cm ceramic tiles, slightly modifying the procedure of Davies (1995). Nubbins were kept in an outdoor, shaded water table until exposed skeleton had healed (13 days). The sex of each coral colony was determined by examining the mesenteries of additional branches for gonad development with a dissecting microscope (C. Hunter, pers. com.). After the healing period, nubbins from each colony were haphazardly assigned to one of the four treatments described below.

Experimental design

The six-week manipulative field experiment took place from July 2 to August 13, 1997. The effects of UVR, water motion, time and coral colony on MAA concentration, photosynthetic pigment concentration and calcification rate were investigated in a factorial-design experiment. There were two levels of UVR treatment: ambient and none, and two levels of water motion: high and low (see "confirmation of treatments" below).
The UVR treatments were imposed in situ by attaching UV-opaque (UVO), and UV-transparent (UVT) Plexiglass filters (30 x 30 cm) to the reef. Threaded stainless steel rods were positioned at 1.5 m depth with marine epoxy (Z-spar), upon which the filters were mounted with stainless bolts and wing nuts approximately 0.3 m above the reef. For the two water motion treatments, corals were transplanted to either the windward side of Coconut Island (high water motion), or to a sheltered cove on the leeward side (low water motion). Thus, nubbins were exposed to one of four treatments: 1) ambient UVR with high water motion (UVT HI), 2) ambient UVR with low water motion (UVT LO), 3) no UVR with high water motion (UVO HI), and 4) no UVR with low water motion (UVO LO). At first, the tiles containing the nubbins were placed on the reef without adhesive, but later marine epoxy was used to fix them to the reef under the Plexiglass plates.

Harvest of the nubbins took place at the start of the experiment, and at three and six weeks. Each nubbin was only measured once for each dependent variable. The experiment was blocked for genetic differences among coral colonies such that the results would be representative of the Coconut Island population of *P. compressa*. In total, there were nine replicates of each of the four treatments (n = 9). Three nubbins from a single colony were placed under each plate: one for harvest at three weeks, one for harvest at six weeks, and one spare in case of loss. In order to test for differences in MAA and photosynthetic pigment concentrations between *P. compressa* colonies naturally occurring at the two sites, five coral nubbins were taken from different colonies at each site on June 15, 1999.
Confirmation of treatments

The optical quality of the UVT and UVO Plexiglass plates was determined using a LiCor® LI-1800UW Underwater scanning spectroradiometer (LiCor®, Lincoln, Nebraska). The instrument was set to scan at 2 nm intervals from 300 to 700 nm. The cosine-corrected sensor was calibrated for both underwater and surface measurement with a LiCor® 1800-02 Optical Radiation Calibrator. Simulating the experimental positioning, the sensor was placed on the reef at 1.5 m while the UVR filters were held 0.3 m above the sensor. During the experiment, the filters were cleaned every morning to remove sediment, invertebrate larvae and algal spores. When necessary, small crustose coralline algae were scrapped off with a non-scratching plastic tool. The filters were scanned before and after the experiment to check for weathering or fouling.

Because Kaneohe Bay is sheltered by a barrier reef system, most water motion found in the Bay is the result of wind-driven waves and tidal fluctuation. Water motion at the two study locations was characterized using the clod card technique described and evaluated in Jokiel and Morrissey (1993) and Thompson and Glenn (1994). Measurements were made during five 24-hour periods during the experiment, placing three replicate clod cards at representative locations at each of the two sites and in a still container control. Measurements were made on days 8, 14, 28, 36, and 42 of the experiment. After the end of the experiment following the removal of the test corals, one clod card was placed under each filter at the two locations (n=18) to characterize water motion under each filter.

Hobo™ temperature meters were placed at both sites for 24 hours at the end of the study period to check for differences in temperature. Surface irradiance was measured
continuously throughout the experiment (Hawaii Institute of Marine Biology weather station) and assumed to be the same at the two sites, which are less than 500 m apart.

*Tissue extraction*

At the time of harvest, coral nubbins were removed from the ceramic tiles, placed in plastic storage bags, and immediately frozen at -50°C. Nubbins were removed from the freezer in batches of ten. The epoxy was quickly pried from the bottom of the nubbin. All manipulations thereafter were conducted in the subdued lighting. Each nubbin was placed in a plastic 50 ml centrifuge tube containing 10 ml of 100% high performance liquid chromatography (HPLC) grade methanol and sonicated in ice water for six minutes. The extracts were then centrifuged at 4°C for two minutes at 1400 x g. The supernatant was decanted and stored at -20°C. Re-extraction occurred twice, adding the new supernatant to the appropriate previous supernatant, resulting in a total extraction volume of 30 ml per nubbin. A 2-ml aliquot of the mixed extract was then transferred into a cryovial, flushed with pure nitrogen gas, flash frozen by dipping the vial into liquid nitrogen, and stored at -80°C until HPLC analysis of photosynthetic pigments was performed. A second aliquot of 1.2 ml was transferred to a microcentrifuge tube and centrifuged at 4000 x g to eliminate particulate matter. The aliquot was then transferred to an autosampler vial and immediately analyzed for MAAs using HPLC.
**HPLC analysis of mycosporine-like amino acids**

The analysis of MAAs was performed using a slightly modified version of the procedures described in Dunlap and Chalker (1986) and Shick et al. (1992). MAAs were separated by reverse-phase isocratic HPLC on a Brownlee RP-8 column (25 cm, 5 μm) protected with a RP-8 guard in an aqueous mobile phase of 0.1% acetic acid and 40% methanol. Peaks were detected by UV-absorbance at 313 and 340 nm, and flow rate was 0.8 ml min⁻¹. Peak identities were confirmed by co-chromatography with authentic, quantitative standards prepared under the guidance of W. C. Dunlap (Australia Institute of Marine Science). Mycosporine-glycine and shinorine standards were isolated from the benthic tunicate, *Lissoclinum patella*, and Asterina-330 from the eye lenses of *Plectropomus leopardus*. Peaks were integrated using EZ-Chrom software, and quantification of the individual MAAs was accomplished using the quantitative standards. MAA concentrations were normalized to nubbin surface area, which was determined using the aluminum foil technique (Marsh 1970).

**HPLC analysis of photosynthetic pigments**

The analysis of photosynthetic pigments was performed using a slightly modified version of the procedure described in Goericke and Repeta (1993). Pigments were separated by reverse-phase HPLC on a Brownlee RP-8 (25 cm, 5 μm) column, protected by an RP-8 guard, using a binary gradient solvent system. Solvents A (MeOH:0.5 N aq. Ammonium acetate, 75:25) and B (MeOH) were programmed on the following gradient (minutes; % solvent A, % solvent B): (0; 100, 0), (20; 0, 100), (25; 0, 100), (33; 100, 0). Absorbance was measured at 440 nm, and flow rate was 1 ml min⁻¹. R. Bidigare
(University of Hawaii) generously supplied purified standards of chlorophyll a (chl a), peridinin and diadinoxanthin. Purified chlorophyll a (chl a) was purchased from Aldrich Chemical Company, Milwaukee, Wisconsin. The crystallized standards were re-hydrated in the appropriate solvent, the absorbance measured at the appropriate wavelength, and the concentration calculated using the published extinction coefficients for the solvent used.

Calcification rate

Coral skeletal growth was quantified by measuring the increase in calcium carbonate skeleton using the buoyant weight technique described in Jokiel et al. (1978). The value for increase in dry weight was cube root transformed into a linear dimension of growth, thus rendering the measurement independent of the corals' original size. This transformation was accomplished using the formula (below) for "mean solid radius" from Maragos (1972). The utility of this transformation is discussed in Buddemeier and Kinzie (1976).

\[ R = 3\sqrt{\frac{3W}{2\pi D}} \]

Where \( R \) = mean solid radius  
\( W \) = net dry weight of aragonite gained per day  
\( D \) = skeletal density of Porites compressa (1.41 g/cm\(^3\))

Statistical analyses

Four-way analysis of variance (ANOVA) was performed on each of the dependent variables using SAS (version 6.12). UVR, water motion, and time were
treated as fixed factors, and colony was treated as a random factor. Originally planned as 
a randomized block design, a less restrictive model was used so that interactions between 
each fixed factor and the random factor could be evaluated. All of the two-way 
interactions among the four factors were included in the model. Thus, the summed mean 
squares of the three-way and the four-way interactions were used as the best estimate of 
error. The unrestricted model III ANOVA (mixed factor levels) was applied, resulting in 
the F-statistics for the main factors being calculated using the mean square of the 
interaction for that factor and the random factor as the denominator (expected mean 
squares reported by SAS). The aptness of the model was evaluated by examining normal 
probability plots and plots of residuals vs. fitted values, residuals vs. each of the four 
factors, and residuals vs. the order in which the samples were analyzed using HPLC. If 
the normality of the data was questionable, Pearson's coefficient of correlation of the 
ordered residuals and their expected values under normality was examined using the 
critical values specified for alpha = 0.05 level of significance (Neter et al. 1990 and 
references therein). If unequal variance was suspected in any of the above plots, the 
Hartley test (for equal sample sizes) or the Bartlett Test (for unequal sample sizes) was 
used as a formal test of the equality of variances. For data sets with unequal variance, an 
ANOVA was performed on the ranks of the observations within each block producing a 
quasi-F statistic, which was evaluated using the F distribution.
RESULTS

Confirmation of treatments

Spectroradiometric measurements revealed that the UVO filters eliminated 93% of the incident UVR between 300 and 400 nm when positioned 0.3 m above where the experimental corals would be at 1.5 m depth. The 7% of incident UVR reaching the sensor was mainly attributable to scattered radiation, because when the filter was held at 1 cm above the sensor, about 98% of UVR was eliminated. Thus, the remaining 2% is probably direct UVR that reaches the sensor through the filter. The UVT filters were transparent to UVR, and had nearly identical spectra to the UVO filters in the visible range (400 – 700 nm). Scanning before and after the experiment confirmed that a negligible amount of filter weathering and fouling had occurred.

The weekly clod card measurements of water motion revealed significant differences between the two sites. The mean difference in the dissolution rate (g day$^{-1}$) of the plaster clod cards between the two sites was 6.48 g day$^{-1}$ (mean significantly above zero, one-sided t-test, p<0.011). On August 25, when measurements were made under all 36 filters at the two sites, dissolution rate was significantly higher at the high water motion site (assuming unequal variance using Satterwaite's approximate t-test, p<0.0001). The variance at the high water motion site was significantly higher (p<0.022) than at the low water site. An approximate transformation of dissolution rate to linear flow rate (cm sec$^{-1}$) was made using a regression equation provided by S. Larned (pers. com.) from a flume calibration of clod cards fabricated using the exact same technique and batch of plaster. The converted values of water motion are 7.1 ± 3.1 cm sec$^{-1}$ at the high water
motion site, and $1.7 \pm 0.2 \text{ cm sec}^{-1}$ at the low water motion site ($n = 5$ days). These estimates are in good agreement with heated mass flow meter measurements made by Lesser et al. (1994) ($7.8 \pm 4.5$ and $3.3 \pm 2.5 \text{ cm sec}^{-1}$ at the high water motion site and low water motion site, respectively).

Other physical parameters were similar at both sites. No significant differences in temperature were detected between the two sites. The flora and fauna at the two sites are similar, with *Porites compressa* and *Montipora verrucosa* as the dominant coral species, and various sessile invertebrates including sabellid worms, zooanthids, and sponges.

*Mycosporine-like amino acids*

Four MAAs were detected in the samples of *Porites compressa*: mycosporine-glycine, asterina-330, shinorine, and an unknown. Asterina-330 occurred at proportionally highest concentrations, closely followed by mycosporine-glycine, with shinorine found at much lower concentrations. If the unknown MAA is assumed to have an extinction coefficient close to those of the known MAAs, then this compound was only found in trace amounts.

The total concentration of MAAs was significantly affected by UVR ($p<0.001$), water motion ($p<0.005$) and time ($p<0.001$) (Fig. 4.1A) (Table 4.1). The total concentration of MAAs decreased in the absence of UVR and stayed near ambient in its presence. The interaction term of Water x Time was significant ($p<0.013$) indicating that the response to water motion changed over time. The UVR x Colony was also significant ($p<0.004$), which indicates that the response to UVR depended upon colony. Colony was an important factor determining MAA concentration of its own right. As this factor was
intended as a random variable, it is not appropriate to test as a main effect. However, the MS is extremely high, indicating that the effect of this block variable was responsible for a large proportion of variance in the model (Table 4.1). Some colonies had higher levels of MAAs than others but they exhibited approximately the same rank in all the treatments.

Each of the individual MAAs responded in a similar fashion to total MAAs (Fig. 4.1). Mycosporine-glycine concentration significantly decreased when UVR was removed (p<0.005) and when water motion was low (p<0.025) (Fig. 4.1B) (Table 4.1). The UVR x Water interaction was significant (p<0.003), indicating that the response to water motion depended upon UVR. There was no effect of water motion when UVR was removed, but in its presence the mean concentration of mycosporine-glycine was higher in high water motion. The UVR x Colony was also significant (p<0.0002), meaning that not all of the nine colonies responded to UVR in the same manner.

UVR (p<0.025), water motion (p<0.025) and time (p<0.005) were significant in determining the concentration of asterina-330, as were the interactions of Water x Time (p<0.01) and UVR x Colony (p<0.008) (Fig. 4.1C) (Table 4.1). These results followed the same pattern as the total MAAs. Two of the colonies had large amounts of asterina-330; these colonies were also the only two females out of the nine colonies.

The data for shinorine were transformed to ranks within blocks because variance was unequal within blocks. The relative amount of shinorine in samples from each colony was quite variable; some colonies had only trace amounts of shinorine, while one colony had about three times the average amount. There was a marked overall decline in concentration of shinorine with time in all treatments (p<0.0001) (Fig. 4.1D) (Table 4.1).
Because the colony effect was removed from the model, there were enough degrees of freedom left to test the three-way interaction UVR x Water x Time, and it was found to be significant (p<0.005). Due to the difficulty in interpreting a three-way interaction, a one-way ANOVA was performed on the rank-transformed data from the six-week harvest, comparing the four treatments. The model was significant (p<0.0001), and Tukey’s Studentized Range Test grouped the two UVO treatments together, while UVT LO was significantly higher than those and lower than the UVT HI treatment (alpha = 0.05).

Coral nubbins from one colony did not contain the unknown MAA. For the eight colonies that did contain this MAA, there was no effect of UVR or water motion on the amount of unknown (as measured in absorbance units). Time was the only important factor in determining the amount of the unknown (p<0.025) (Table 4.1), apparent as a decrease over time.

Coral nubbins taken from colonies of *P. compressa* occurring naturally at the two water motion sites (n = 5) did not show any significant differences for total MAA concentration (paired t-test, p<0.57), or any of the individual MAAs (p-values all greater than 0.15).
Table 4.1. Results of four-way ANOVA for MAA concentrations including total MAAs, mycosporine-glycine, asterina-330, shinorine, and the unknown. Results based upon the SAS Institute's general linear model procedure for mixed models, using the appropriate denominator to calculate F according to the expected mean squares reported by SAS with the appropriate degrees of freedom. “Colony” was treated as a random variable. “Shinorine” data were rank-transformed, ranking the observations within block (“colony”). MS = mean square; na = not applicable. P-values less than 0.05 are highlighted in bold.

<table>
<thead>
<tr>
<th>Dependent Variable</th>
<th>UVR</th>
<th>Water</th>
<th>Time</th>
<th>Colony</th>
<th>UVR x Time</th>
<th>UVR x Water</th>
<th>Water x Time</th>
<th>Colony x UVR</th>
<th>Colony x Water</th>
<th>Colony x Error</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total MAAs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>df</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>8</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>33</td>
</tr>
<tr>
<td>MS</td>
<td>12103</td>
<td>2030</td>
<td>3055</td>
<td>2631</td>
<td>475.8</td>
<td>339.3</td>
<td>802.7</td>
<td>80.62</td>
<td>422.19</td>
<td>123.8</td>
<td>116.5</td>
</tr>
<tr>
<td>F</td>
<td>28.67</td>
<td>16.39</td>
<td>26.22</td>
<td>na</td>
<td>4.09</td>
<td>2.91</td>
<td>6.89</td>
<td>0.69</td>
<td>3.63</td>
<td>1.06</td>
<td></td>
</tr>
<tr>
<td>p</td>
<td>&lt;0.001</td>
<td>&lt;0.005</td>
<td>&lt;0.001</td>
<td>na</td>
<td>&lt;0.052</td>
<td>&lt;0.98</td>
<td>&lt;0.013</td>
<td>&gt;0.10</td>
<td>&lt;0.004</td>
<td>&gt;0.10</td>
<td></td>
</tr>
<tr>
<td>Mycosporine-gly</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>df</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>8</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>33</td>
</tr>
<tr>
<td>MS</td>
<td>2496</td>
<td>161.3</td>
<td>92.63</td>
<td>765.4</td>
<td>65.05</td>
<td>209.5</td>
<td>52.13</td>
<td>17.72</td>
<td>115.7</td>
<td>18.06</td>
<td>20.64</td>
</tr>
<tr>
<td>F</td>
<td>21.57</td>
<td>8.93</td>
<td>4.49</td>
<td>na</td>
<td>3.15</td>
<td>10.15</td>
<td>2.53</td>
<td>0.86</td>
<td>5.61</td>
<td>0.87</td>
<td></td>
</tr>
<tr>
<td>p</td>
<td>&lt;0.005</td>
<td>&lt;0.025</td>
<td>&gt;0.10</td>
<td>na</td>
<td>&lt;0.085</td>
<td>&lt;0.003</td>
<td>&gt;0.10</td>
<td>&gt;0.10</td>
<td>&lt;0.002</td>
<td>&gt;0.10</td>
<td></td>
</tr>
<tr>
<td>Asterina-330</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>df</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>8</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>33</td>
</tr>
<tr>
<td>MS</td>
<td>1292</td>
<td>622.7</td>
<td>856.2</td>
<td>2878</td>
<td>172.9</td>
<td>2.26</td>
<td>339.8</td>
<td>55.18</td>
<td>148.6</td>
<td>74.06</td>
<td>45.30</td>
</tr>
<tr>
<td>F</td>
<td>8.70</td>
<td>8.41</td>
<td>15.52</td>
<td>na</td>
<td>3.82</td>
<td>0.05</td>
<td>7.5</td>
<td>1.22</td>
<td>3.28</td>
<td>1.63</td>
<td></td>
</tr>
<tr>
<td>p</td>
<td>&lt;0.025</td>
<td>&lt;0.025</td>
<td>&lt;0.005</td>
<td>na</td>
<td>&lt;0.060</td>
<td>&gt;0.10</td>
<td>&lt;0.010</td>
<td>&gt;0.10</td>
<td>&lt;0.008</td>
<td>&gt;0.1</td>
<td></td>
</tr>
<tr>
<td>Shinorine (rank)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>df</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>na</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>na</td>
<td>na</td>
<td>1*</td>
<td>64</td>
</tr>
<tr>
<td>MS</td>
<td>213.6</td>
<td>20.06</td>
<td>76.06</td>
<td>na</td>
<td>1.389</td>
<td>2.722</td>
<td>2.722</td>
<td>na</td>
<td>na</td>
<td>6.722*</td>
<td>0.778</td>
</tr>
<tr>
<td>F</td>
<td>274.6</td>
<td>25.79</td>
<td>97.79</td>
<td>na</td>
<td>1.79</td>
<td>3.50</td>
<td>3.50</td>
<td>na</td>
<td>na</td>
<td>8.64*</td>
<td></td>
</tr>
<tr>
<td>p</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>na</td>
<td>&gt;0.10</td>
<td>&lt;0.066</td>
<td>&lt;0.066</td>
<td>na</td>
<td>na</td>
<td>&lt;0.005*</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>df</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>7</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>29</td>
</tr>
<tr>
<td>MS</td>
<td>131578</td>
<td>2737307</td>
<td>109909260</td>
<td>36233313</td>
<td>548253</td>
<td>5519</td>
<td>1563617</td>
<td>947594</td>
<td>638389</td>
<td>1055106</td>
<td>724348</td>
</tr>
<tr>
<td>F</td>
<td>0.14</td>
<td>3.78</td>
<td>10.34</td>
<td>na</td>
<td>0.76</td>
<td>0.01</td>
<td>2.16</td>
<td>1.31</td>
<td>0.88</td>
<td>1.46</td>
<td></td>
</tr>
<tr>
<td>p</td>
<td>&gt;0.10</td>
<td>&lt;0.062</td>
<td>&lt;0.025</td>
<td>na</td>
<td>&gt;0.10</td>
<td>&gt;0.10</td>
<td>&gt;0.10</td>
<td>&gt;0.10</td>
<td>&gt;0.10</td>
<td>&gt;0.10</td>
<td></td>
</tr>
</tbody>
</table>

* These values refer to the three-way interaction of UVR x Water x Time.
Fig. 4.1. Mean concentrations of total and individual MAAs during the six-week field experiment with *Porites compressa*. (A) Total MAAS, (B) Mycosporine-glycine, (C) Asterina-330, (D) Shinorine. The four treatments were designated as follows: UVT HI = UV present at high water motion, UVT LO = UV present at low water motion, UVO HI = UV absent at high water motion, UVO LO = UV absent at low water motion. Time = zero represents the mean value for the nine colonies at their ambient state before the treatments were imposed (these data were not included in the ANOVA). Error bars are equal to one standard error.
Photosynthetic pigments

Four main photosynthetic pigments were detected in the tissues of *P. compressa*: chl a, peridinin, chl c2, and diadinoxanthin (in order of decreasing prevalence). Even after samples were stored for one year at -50°C, levels of degradation products evident in the chromatograms were small. All of the pigment data were transformed to ranks within blocks as variance was unequal within blocks.

There was no effect of UVR on total photosynthetic pigment concentration (p>0.1) (Table 4.2) (Fig. 4.2). Water motion, however, was quite important in determining total pigment concentration (p<0.0001 for total pigments and all individual pigments). The concentrations of all four pigments were higher in high water motion than in low water motion. Time was also a significant determinant of pigment concentration (p<0.03 for all pigments except peridinin p<0.11). This effect was evident as a slight decrease observed in all treatments during the first three weeks, and then a large increase in the high water motion corals and negligible change in the low water motion corals during the second three weeks (Fig. 4.2 to 4.3). Although there was no significant effect of UVR for any of the pigments, the UVO HI treatment had the highest mean concentration for all pigments (Fig. 4.2 to 4.3).

Coral nubbins taken from *P. compressa* colonies occurring naturally at the high water motion site had significantly higher total photosynthetic pigment concentrations than those from the low water motion site (paired t-test, n = 5, p<0.011). This difference was true for all four of the individual pigments as well (all p-values less than 0.025). The mean total pigment concentration was 31.0 μg cm⁻² ± 4.7 SE at the high water motion site, and 21.2 ± 4.8 μg cm⁻² SE at the low water motion site.
Table 4.2. Results of four-way ANOVA on rank-transformed data for pigment concentrations including total pigments, chlorophyll $a$, chlorophyll $c_2$, peridinin, and diadinoxanthin, plus calcification rate. Results based upon the SAS Institute's general linear model procedure, using the reported F value as the estimate of the quasi-F statistic. MS = mean square. P-values less than 0.05 are highlighted in bold.

<table>
<thead>
<tr>
<th>Dependent Variable</th>
<th>UVR</th>
<th>Water</th>
<th>Time</th>
<th>UVR x Time</th>
<th>UVR x Water</th>
<th>Water x Time</th>
<th>UVR x Water x Time</th>
<th>Error</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Pigments</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$df$</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>64</td>
</tr>
<tr>
<td>MS</td>
<td>2.722</td>
<td>112.5</td>
<td>34.72</td>
<td>2.000</td>
<td>2.000</td>
<td>8.000</td>
<td>6.722</td>
<td>3.271</td>
<td>71</td>
</tr>
<tr>
<td>Quasi-F</td>
<td>0.83</td>
<td>34.39</td>
<td>10.62</td>
<td>0.61</td>
<td>0.61</td>
<td>2.45</td>
<td>2.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$p$</td>
<td>&gt;0.10</td>
<td>&lt;0.0001</td>
<td>&lt;0.0018</td>
<td>&gt;0.10</td>
<td>&gt;0.10</td>
<td>&gt;0.10</td>
<td>&gt;0.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlorophyll $a$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$df$</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>64</td>
</tr>
<tr>
<td>MS</td>
<td>0.000</td>
<td>122.7</td>
<td>50.00</td>
<td>0.222</td>
<td>1.389</td>
<td>6.722</td>
<td>2.722</td>
<td>3.035</td>
<td>71</td>
</tr>
<tr>
<td>Quasi-F</td>
<td>0.000</td>
<td>40.44</td>
<td>16.48</td>
<td>0.07</td>
<td>0.46</td>
<td>2.22</td>
<td>0.90</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$p$</td>
<td>&gt;0.10</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&gt;0.10</td>
<td>&gt;0.10</td>
<td>&gt;0.10</td>
<td>&gt;0.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlorophyll $c_2$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$df$</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>64</td>
</tr>
<tr>
<td>MS</td>
<td>2.000</td>
<td>93.39</td>
<td>20.06</td>
<td>2.722</td>
<td>2.722</td>
<td>3.556</td>
<td>2.000</td>
<td>3.931</td>
<td>71</td>
</tr>
<tr>
<td>Quasi-F</td>
<td>0.51</td>
<td>23.76</td>
<td>5.10</td>
<td>0.69</td>
<td>0.69</td>
<td>0.90</td>
<td>0.51</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$p$</td>
<td>&gt;0.10</td>
<td>&lt;0.0001</td>
<td>&lt;0.028</td>
<td>&gt;0.10</td>
<td>&gt;0.10</td>
<td>&gt;0.10</td>
<td>&gt;0.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peridinin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$df$</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>64</td>
</tr>
<tr>
<td>MS</td>
<td>8.000</td>
<td>84.50</td>
<td>10.89</td>
<td>0.222</td>
<td>0.056</td>
<td>9.389</td>
<td>4.500</td>
<td>4.069</td>
<td>71</td>
</tr>
<tr>
<td>Quasi-F</td>
<td>1.97</td>
<td>20.76</td>
<td>2.68</td>
<td>0.05</td>
<td>0.01</td>
<td>2.31</td>
<td>1.11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$p$</td>
<td>&gt;0.10</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&gt;0.10</td>
<td>&gt;0.10</td>
<td>&gt;0.10</td>
<td>&gt;0.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diadinoxanthin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$df$</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>64</td>
</tr>
<tr>
<td>MS</td>
<td>5.556</td>
<td>64.22</td>
<td>43.56</td>
<td>0.000</td>
<td>0.889</td>
<td>10.89</td>
<td>3.556</td>
<td>3.896</td>
<td>71</td>
</tr>
<tr>
<td>Quasi-F</td>
<td>1.43</td>
<td>16.48</td>
<td>11.18</td>
<td>0.000</td>
<td>0.23</td>
<td>2.80</td>
<td>0.91</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$p$</td>
<td>&gt;0.10</td>
<td>&lt;0.0001</td>
<td>&lt;0.0014</td>
<td>&gt;0.10</td>
<td>&gt;0.10</td>
<td>&lt;0.0994</td>
<td>&gt;0.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcification rate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$df$</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>61</td>
</tr>
<tr>
<td>MS</td>
<td>0.401</td>
<td>100.4</td>
<td>8.459</td>
<td>2.242</td>
<td>3.948</td>
<td>0.030</td>
<td>0.100</td>
<td>3.642</td>
<td>68</td>
</tr>
<tr>
<td>Quasi-F</td>
<td>0.11</td>
<td>27.58</td>
<td>2.32</td>
<td>0.62</td>
<td>1.08</td>
<td>0.01</td>
<td>0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$p$</td>
<td>&gt;0.10</td>
<td>&lt;0.0001</td>
<td>&lt;0.10</td>
<td>&gt;0.10</td>
<td>&gt;0.10</td>
<td>&gt;0.10</td>
<td>&gt;0.10</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Fig. 4.2. Mean concentration of total photosynthetic pigments for the nine colonies during the six-week field experiment with *Porites compressa*. The four treatments were designated as follows: UVT HI = UV present at high water motion, UVT LO = UV present at low water motion, UVO HI = UV absent at high water motion, UVO LO = UV absent at low water motion. Time = zero represents the mean value for the nine colonies at their ambient state before the treatments were imposed (these data were not included in the ANOVA). Error bars are equal to one standard error.
Fig. 4.3. Mean concentrations of individual photosynthetic pigments during the six-week field experiment with *Porites compressa*. (A) Chlorophyll $a$, (B) Diadinoxanthin, (C) Chlorophyll $c_2$, (D) Peridinin. Treatments are the same as in the previous figure. Error bars equal one standard error.
Calcification rate

Water motion was the only significant factor in determining calcification rate of the coral nubbins \( p<0.0001 \) (Table 4.2) (Fig. 4.4). Calcification rate was higher for the corals at the high water motion site than for those at the low water motion site during both halves of the experiment (Fig. 4.4). The calcification rate data were transformed to ranks within blocks as variance was unequal within block. Corals did not calcify at a faster rate in the absence of UVR than in its presence.
Fig. 4.3. Mean calcification rate for the nine colonies during the six-week field experiment with *Porites compressa*. The four treatments were designated as follows: UVT HI = UV present at high water motion, UVT LO = UV present at low water motion, UVO HI = UV absent at high water motion, UVO LO = UV absent at low water motion. Error bars equal one standard error.
DISCUSSION

*Mycosporine-like amino acids*

Ultraviolet radiation was a significant determinant of MAA concentration for nubbins of *Porites compressa*, but not the only one (Fig. 4.1). Water motion and time (duration of treatment) also had significant effects on MAA concentration (Fig. 4.1). When UVR was screened from the coral’s environment, MAA concentration decreased regardless of water motion. However, when UVR was present, MAA concentration was higher in high water motion than in low water motion. These results suggest that high water motion and ambient levels of UVR are necessary for the upkeep of MAAs if the coral has been living in these conditions. This could have been a reflection of stress induced upon the low water motion corals because of being transplanted to a water motion regime to which they were not acclimated. However, it could be an indication that MAA production is a mass transfer limited process. In coral, the diffusive boundary layer decreases in thickness with increasing water motion, leading to higher rates of uptake of needed substrates (Atkinson and Bilger 1992; Lesser et al. 1994). It has been shown that corals can acclimated to different flow regimes when transplanted by modifying their growth form (Maragos 1972). Perhaps if given long enough, the nubbins would have acclimated to the new flow regime and regained levels of MAAs appropriate to the UVR regime. Support for this idea is in the fact that the control nubbins from *P. compressa* colonies naturally occurring at the two sites did not show significant differences in MAA concentrations.

Unexpectedly, different colonies of *P. compressa* responded in an individualistic manner to changes in UVR environment. Certain colonies had consistently higher
concentrations of mycosporine-glycine, and low amounts of asterina-330, while in some the pattern was reversed. The two female colonies had the highest concentrations of asterina-330, as well as the highest total MAA concentrations. The experiment was performed in mid summer, which corresponds with the spawning season for this species (spawning was observed in two male colonies during the healing period before the experiment commenced). Perhaps MAAs were being packaged into the oocytes before spawning, resulting in elevated concentrations compared to the males. One colony was almost completely devoid of the unknown MAA (only one sample had trace amounts). Another colony had about three times more shinorine compared to the other colonies, while two of the colonies had very little shinorine. These results are congruent with the observation made by Hunter (1985) that colonies of different immunohistocompatibility, which corresponded to morphotype as determined by color, branch width, etc., had different chromatogram “signatures” using the same HPLC techniques as in this study.

Photosynthetic pigments

This study showed that the coral-algal symbiosis responded to changes in water motion regime by decreasing concentrations of photosynthetic pigments in low water motion (Fig. 4.2 to 4.3). This sensitivity to decreases in water motion could be a response to an increase in the diffusive boundary layer thickness, resulting in a decrease in zooxanthellae number and/or the amount of pigment per zooxanthella. Total pigment concentration was not sensitive, as hypothesized, to UVR. This was surprising because UVR effects have been shown in previous studies. Increases in UVR resulting from transplantation from deep to shallow depths cause a decrease in chl a concentration in the
brown morph of *Porites asteroides* (Gleason 1993) and in *Montipora verrucosa* (Grottioli and Kuffner 1995). Perhaps an increase in UVR, as opposed to a reduction, is necessary to observe the UVR effect.

It is interesting to note that variance within blocks (colonies) was unequal for all of the pigment data; individual colonies responded differently to water motion. These differences in responsiveness could have resulted from micro-scale differences in water motion experienced by the original colonies from which the nubbins were taken. The original site of the coral colonies was not meant to be a factor in the experiment, but in hindsight it may have been important. The water flow regimes were not quantified at the original sites, but they were probably closer in the water motion regime to the high water motion site than the low water motion site. Thus, the high water motion treatment probably represented a continuation of ambient conditions or perhaps a slight increase rather than a ‘treatment.’

The significant water motion effect in this experiment could be a reflection of the fact that the nubbins placed in low water motion were not acclimated to this flow regime, rather than a true difference in the state of the coral-algal symbiosis when it is found in that habitat. The slight decrease in concentration of pigments in all nubbins seen after the first three weeks suggests that they may have been responding to stress associated with being separated from the original colony and/or the treatment manipulations. However, the control nubbins naturally occurring at the two sites showed that pigment concentrations were naturally and significantly higher at the high water motion site in comparison with the low water motion site.
Calcification rate

UVR did not affect the calcification rate of *P. compressa* nubbins (Fig. 4.4). Mitigating the effects of UVR and maintaining high levels of MAAs did not procure a cost, as measured by skeletal growth, to the nubbins. Other researchers have reported unusually high growth rates for *Pocillopora damicornis* in Hawaii when UVR was screened out (Jokiel and York 1982). Gleason and Wellington (1993) found that the brown morph, but not the green morph, of *Porites asteroides* in the Caribbean had lower linear extension rates when transplanted from 6 m to 1 m depth when UVR was present compared to in its absence. These results suggest that some species and/or morphs of coral may be more adapted to ambient levels of UVR than others.

Calcification rate was significantly lower for corals at the low water motion site than at the high water motion site (Fig. 4.4). Previous studies have found that high water motion results in increased calcification rate for three species of coral transplanted from the reef to outdoor aquaria (Jokiel 1978; Dennison and Barnes 1988). However, Harriott (1998) showed that for *Acropora formosa* in the field, calcification rate between corals at a wave-exposed site and a protected site did not differ, but linear extension was significantly greater at the protected site (i.e., wave-protected corals were less dense). Even though the present field study was conducted for six weeks, the corals may have been still acclimatizing to the new location. If the experiment had been continued, perhaps the calcification rates at the two sites would have converged.

The original location of the coral colonies may have affected the calcification response to the water motion treatment, as it probably did the photosynthetic pigments. It is not known whether the slower calcification rate was due to stress related to corals.
being relocated to a water motion regime in which they were not acclimated to or if this species actually calcifies slower in low water motion habitats. *P. compressa* is quite common at both sites. The fact that photosynthetic pigments normalized to surface area are significantly lower in corals naturally occurring at the low water motion site compared to the high water motion site suggests that calcification could be naturally lower at the low water motion site.

**Conclusions**

The results of this study indicate that *P. compressa* seems to be adapted to ambient levels of UVR. MAA concentration in *P. compressa* nubbin tissues was largely determined by UVR, and nubbins did not deposit skeleton at a faster rate nor did they acquire more photosynthetic pigments in the absence of UVR. However, corals exposed to UVR at the low water motion site were not able to keep MAA concentrations as high as those exposed to UVR at the high water motion site. The production of MAAs may represent a short-term cost with respect to the nitrogen pool available to the zooxanthellae when nitrogen is limited because of changes in the diffusive boundary layer.

The significant water motion effects on MAA concentration, photosynthetic pigments and calcification rate revealed in this study have implications for transplantation of reef corals. Mitigation laws requiring transplantation of corals when coastline development involves impacting coral reefs may become important in the near future as the economic importance of reefs is beginning to be addressed (P. L. Jokiel, pers. com.). Also, reefs around the world are being degraded at an alarming rate (Hoegh-
Guldberg 1999), and rehabilitation projects will need to know what factors affect the acclimation of corals to new environments. Cross transplantation experiments would be a useful next step in this research to establish how long corals need to acclimate to a change in water motion and to establish transplantation limits.
CHAPTER 5: THE EFFECTS OF ULTRAVIOLET RADIATION ON LARVAL RECRUITMENT OF THE REEF CORAL, Pocillopora damicornis Linnaeus

INTRODUCTION

Coral reefs around the world are degrading at an alarming rate (Hoegh-Guldberg 1999). Understanding why this is so, and then attempting management techniques to rehabilitate degraded reefs, depends upon a clear understanding of coral reproductive ecology. Reef rejuvenation is dependent upon the establishment of juvenile corals, which in turn depends upon adult colony fecundity, fertilization success, larval transport, settlement, metamorphosis (recruitment) and the survival of new recruits.

The physical factors reported to negatively affect coral recruitment include sedimentation (Hodgson 1990; Babcock and Davies 1991), eutrophication (Tomascik 1991), and solar irradiance in the visible region (Maida et al. 1994; Babcock and Mundy 1996). Often ignored in recruitment studies, ultraviolet radiation (UVR, 280 – 400 nm) is an important environmental factor that can have profound effects on aquatic organisms (Calkins and Thordardottir 1980; Holm-Hansen et al. 1993). Only in the past few decades has UVR been considered important in structuring coral reef communities (Jokiel 1980), probably because of the misconception that UVR is absorbed rapidly in the water column. UVR can penetrate to depths of 30 m in tropical, oligotrophic waters (Fleischmann 1989). This often-overlooked parameter has not been examined for potential effects on coral recruitment in the UV-transparent waters of the tropical Pacific.
The negative effect of UV-B radiation (280 – 320 nm) on coral larvae survivorship has been demonstrated for *Agaricia agaricites* in the Caribbean (Gleason and Wellington 1995). Larvae originating from depths of 24 m had higher mortality as a result of UV-B radiation than those from 3 m depth. This difference was attributed to the three-fold higher concentration of mycosporine-like amino acids (MAAs) in the larvae from 3 m compared to those from 24 m depth. MAAs, putative ‘sunscreen’ molecules found in the tissues of many marine organisms (see Dunlap and Shick 1998 for review), are presumably produced by the zooxanthellae via the shikimate pathway unique to photosynthetic organisms (Bentley 1990). A photoprotective role has been ascribed to MAAs based upon the absorbance properties of the molecules ($\lambda_{\text{max}}$ ranging from 309 to 360 nm) and studies correlating MAA concentration to UVR exposure (Dunlap and Shick 1998).

The aim of this study was to examine the effects of UVR on survivorship and recruitment success of the Indo-Pacific reef coral, *Pocillopora damicornis*. Larvae were taken from adults of four origins: shallow (<0.5 m), deep (2 – 3 m), incubated in ambient UVR, and incubated in no UVR, and then tested for their sensitivity to UVR in three factorial field experiments. It was hypothesized that UVR would have negative effects on larvae from deep adults and from adults incubated in the absence of UVR because these larvae would be expected to have lower concentrations of MAAs.
MATERIALS AND METHODS

Collection and maintenance of corals

Colonies of *Pocillopora damicornis* (Linnaeus) were collected from a variety of locations throughout Kaneohe Bay, Hawai'i on May 23, 1998. The colonies were transported to the Hawaii Institute of Marine Biology (HIMB) where four colonies (one from each location) were placed in each of six outdoor aquaria supplied with continuously flowing seawater. Three aquaria were covered with UV-opaque (UVO) and three with UV-transparent (UVT) Plexiglas filters (described in Jokiel and York 1982), resulting in an exposure time of 51 days before larvae were obtained for Experiment I (see below).

‘Deep’ and ‘shallow’ corals were collected from Kaneohe Bay on September 9, 1998. Six colonies were collected from a depth of 2 to 3 m at reef “No. 42,” and six from the reef flat (<0.5 m) of “Checker” reef. Colonies were transported back to HIMB and immediately placed in planulae collection containers described by Jokiel et al. (1985).

Larval recruitment chambers

Larval recruitment chambers were constructed of 15.2 cm long halves of 15.2 cm diam. transparent polyvinylchloride (PVC) tubing with a plate of either UV-transparent (UVT) or UV-opaque (UVO) Plexiglas attached to the top (Fig. 5.1). The transparent PVC tubing was of the same optical quality as the UVT filters (transparent to 95% of ambient UVR and 98% of visible light). Mesh of 183 μm was adhered with a glue gun to the sides of the chambers, allowing maximum water circulation while keeping the larvae
from escaping. The bottoms of the chambers were secured to 30 x 30 cm terracotta tiles via 10 x 10 cm Velcro™ patches, providing secure platforms for the chambers when placed on the reef. A 1/4” port was drilled into the side of each chamber so that the larvae could be placed inside, and the hole then covered with vinyl electrical tape. A biologically conditioned terracotta recruitment tile approximately 10 x 10 x 1 cm was placed in each chamber and attached with plastic clay (‘plasticine’). During Experiments I and III, the space underneath the tile was entirely filled with plasticine. During Experiment II, the space was left available to the larvae.

Measurements of the spectral irradiance reaching the inside of the chambers were taken with a LiCor® LI-1800UW underwater scanning spectroradiometer (LiCor®, Lincoln, Nebraska) at 0.5 m depth on the reef. One of each type of chamber (UVO and UVT) was modified to fit over the sensor in order to measure the amount and quality of irradiance falling on the recruitment tile of an intact chamber.
Fig. 5.1. Larval recruitment chamber. Mesh size = 183 \mu m.
Planula larvae

On the day of the full moon, colonies were moved from the UVR incubation tanks or directly from the field to individual larvae collection containers. Larvae were released mainly during the day, and were collected from the containers the following morning. All larvae from adults of like treatment (deep, shallow, UVT or UVO) were combined. Larvae were counted under a dissecting microscope and placed into glass scintillation vials until they were put into the recruitment chambers. One hundred larvae put in each chamber using a large-mouthed, plastic pipette. The chambers were placed at a protected reef site in the lee of Coconut Island at 0.5 m depth for one week, and cleaned of sediment each morning.

Scoring of recruitment tiles

At the end of one week, chambers were transported in seawater back to HIMB and temporarily placed in a water table while waiting to be examined. A recruit was defined as a larva that had undergone metamorphosis with evidence of skeletal calcification. Larvae attached by sticky threads to the substrate were not considered recruits. Larvae were scored according to recruitment location: tile top, tile sides, tile underside (available in experiment II only), mesh sides, chamber surface exposed to direct irradiance, and chamber surface shaded from direct irradiance. Later, these categories were merged into two groups: those exposed to direct sun (tile top plus exposed chamber surface) and those in cryptic or shaded locations (tile sides, mesh, tile bottom and shaded chamber surface). The water inside the chamber was poured through a 183 μm mesh sieve to count the larvae still swimming in the water column. Total
survival was calculated by adding the number of recruits and the number of swimming larvae.

Experimental design

The purpose of Experiment I was to examine the sensitivity of planulae from adult colonies incubated in ambient UVR (UVT) and in the absence of UV (UVO) to UVR exposure. The spaces under the recruitment tiles were filled with plasticine so that the larvae could not escape direct UVR. The planulae were collected from adult colonies on July 13, and placed in chambers on the reef from July 15 to 22. The recruitment tiles had been biologically conditioned for 30 days in a shaded, outdoor water table and were uniformly covered by small amounts of crustose and turf algae.

The purpose of Experiment II was to compare the UVR sensitivity of planulae released from shallow (<0.5 m) and deep (2 – 3 m) adults. Also, the space under the recruitment tile was left available to the larvae in order to see if the location of recruitment (shade vs. direct sun) would be affected by UVR. Larvae were spawned from adult colonies on September 9, and placed in chambers on the reef from September 11 to 18. The recruitment tiles had been conditioned for 80 days in an outdoor sea table in the shade. Patches of crustose coralline algae (CCA) were distributed preferentially to one face of each tile. The CCA-covered surfaces were all faced down so that the orientation was consistent in all chambers.

Experiment III also tested the UVR sensitivity of larvae from shallow and deep, but the spaces under the tiles were filled so that the larvae could not escape the UVR. Larvae released on September 10 by the same colonies used in Experiment II were placed
in chambers on the reef from September 12 to 19. The recruitment tiles had been conditioned for 81 days in the same shaded water table mentioned above. CCA was patchily distributed over the tiles.

High Performance Liquid Chromatography (HPLC) of MAAs

At the time the larvae were being counted for placement into the larval recruitment chambers, samples were taken for analysis of MAA concentration. Batches of twenty UVO/UVT or ten deep/shallow larvae were counted and immediately frozen at \(-50^\circ\) C until the time of extraction (\(n=5\) batches). On May 21-22, 1999, larvae were extracted in 100 \(\mu\)l of HPLC grade methanol, and sonicated for 15 minutes in an ice bath. The methanol extracts were centrifuged for 3 minutes at 4000 x g, and the supernatant drawn off and transferred to a polypropylene, low-volume insert placed inside an autosampler vial until analysis.

Adult tissue samples were taken from three colonies of each larval origin (deep, shallow, UVO, UVT) at the time of planula release for analysis of MAAs. The samples were extracted in 20 ml HPLC grade methanol, and sonicated on ice for 12 minutes and centrifuged at 1400 x g for 2 minutes.

MAA analysis was performed by slightly modifying the procedures described in Dunlap and Chalker (1986) and Shick et al. (1992). Using a Shimadzu HPLC system MAAs were separated by reverse-phase isocratic HPLC on a Brownlee RP-8 column (25 cm, 5 \(\mu\)m) protected with a RP-8 guard, in an aqueous mobile phase of 0.1% acetic acid and 40% methanol. The flow rate was 0.8 ml min\(^{-1}\), and the detection of peaks was by UV absorbance at 313 and 340 nm. Identities of peaks were confirmed by co-
chromatography with authentic, quantitative standards prepared by the author under the
guidance of W. C. Dunlap (Australia Institute of Marine Science). Peaks were integrated
using EZ-Chrom™ software, and quantification of the individual MAAs was
accomplished by calibration with quantitative standards. MAA concentrations were
expressed either per larva or per gram dry skeletal weight.

Statistical analysis

Model I (fixed factor) two-way ANOVA was performed for each of the three
experiments using SAS (version 6.12). The model included the two factors, UV
treatment and origin of the larvae, plus the interaction term. When sample sizes were
unequal (as in Experiment II), the Type III sums of squares reported by SAS were used as
they assign equal importance to each treatment mean (Littell et al. 1991). The aptness of
the ANOVA model was evaluated by examining normal probability plots, and plots of
residuals versus fitted values and each of the two factors.
RESULTS

*Larval recruitment chambers*

The results of the spectral irradiance measurements for a UVT and a UVO chamber are presented in Fig. 5.2. Approximately 91% of the irradiance in the UV portion (300 to 400 nm) measured in the UVT chamber was eliminated in the UVO chamber. The jagged appearance of the spectral scan resulted from the focusing of light by wavelets passing over the sensor.

*Experiment I – UVT/UVO larvae, tiles closed*

Survival of larvae averaged 70% in all treatments (Table 5.1) (Fig. 5.3A). In one chamber there was an infestation of juvenile gastropods, which had grazed the live tissues from the skeletons by the time of analysis. Because the mortality occurred after recruitment, empty skeletons and live recruits were combined for the estimate of total recruitment for this experiment.

There was a highly significant effect of UVR on recruitment (p<0.0059) (Table 5.2) (Fig. 5.3A). UVR appears to have had a slight negative effect on survival, but this was not statistically significant (p<0.0655) (Fig. 5.3A) (Table 5.2). Larval origin was not important in determining either larval survival or recruitment (Table 5.2) (Fig. 5.3A).

The proportion of recruits located in spaces exposed to direct sunlight was not significantly greater in any of the treatments (arcsine transformed) (Table 5.2).
Experiment II – Deep/Shallow, tiles open

Total survival of larvae was high in all treatments, averaging 69% except in two of the chambers in which very few survivors were found (Table 5.1). One replicate from the deep/UVT and one replicate from shallow/UVO treatment were affected. These data were removed from the analysis on the basis that something other than treatment effects caused the death of all the larvae before recruitment occurred.

Total recruitment was negatively affected by UVR (p<0.0012) (Fig. 5.3B) (Table 5.2). For survival, there was a significant interaction between the effects of UVR and larval origin (p<0.0137) (Table 2). Multiple comparisons using Tukey’s Honest Significant Difference test (alpha = 0.05 experiment-wise error rate) revealed that shallow larvae not exposed to UV (UVO) showed significantly higher survival than shallow larvae exposed to UV (UVT).

In this experiment, the underside of the tiles was left accessible to the larvae. The proportion of larvae that settled cryptically or in shade (including under the tile) was in general much greater in this experiment than in Experiment I (Table 5.1). However, there were more patches of crustose coralline algae (CCA) on the undersides of the tiles than on the tops. Larvae were found directly attached to patches of CCA. As CCA may have been a confounding factor, the data for location of recruitment were not statistically analyzed.
Experiment III – Deep/Shallow larvae, tiles closed

In this experiment, survival was universally low, averaging only 26% (Fig. 5.3C) (Table 5.1). However, it appeared that mortality ensued after recruitment had occurred because fully formed skeletons were abundant on the tiles. Live recruits and empty skeletons were combined for the estimation of total recruitment. Since survival was probably not a result of the treatment effects in this experiment, it was not statistically analyzed.

Recruitment was negatively affected by the presence of UVR \( (p<0.0315) \) (Fig. 5.3C) (Table 5.2). The proportion of recruits located in direct sun was affected by larval origin (arcsine transformed) \( (p<0.05) \) (Table 5.2), with deep larvae showing a preference for directly light places. The proportion of recruits found directly exposed to the sun also seemed to be affected by UVR, with more recruitment in direct sun in the absence of UVR (UVO treatment) than in its presence (UVT treatment), although this effect was marginally significant \( (p<0.06) \) (Table 5.2).
Fig. 5.2. Spectral irradiance scans (Watts m$^{-2}$ nm$^{-1}$) inside of a UVT and a UVO larval recruitment chamber placed at 0.5 m depth on the reef using a LiCor LI-1800UW scanning spectroradiometer.
Table 5.1 *Pocillopora damicornis*. Fate of one hundred larvae per recruitment chamber. There were four replicates per treatment in all cases except where marked (*) n = 3. UVO = UV-opaque, UVT = UV-transparent. Values are means (standard error). N/a = not available.

<table>
<thead>
<tr>
<th>Origin and UVR Treatment</th>
<th>Total survival (%)</th>
<th>Total recruitment (%)</th>
<th>Recruits in direct sun (%)</th>
<th>% recruits in direct sun</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experiment I</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UVO in UVT</td>
<td>65.0(3.1)</td>
<td>51.3(4.9)</td>
<td>20.5(1.6)</td>
<td>41(5.4)</td>
</tr>
<tr>
<td>UVT in UVT</td>
<td>65.5(4.3)</td>
<td>44.5(4.1)</td>
<td>18.3(6.4)</td>
<td>41(12)</td>
</tr>
<tr>
<td>UVO in UVO</td>
<td>71.0(4.8)</td>
<td>66.0(5.4)</td>
<td>31.8(2.6)</td>
<td>50(6.3)</td>
</tr>
<tr>
<td>UVT in UVO</td>
<td>79.0(6.4)</td>
<td>63.0(5.4)</td>
<td>35.3(3.7)</td>
<td>56(4.7)</td>
</tr>
<tr>
<td><strong>Experiment II</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deep in UVT*</td>
<td>69.7(3.7)</td>
<td>41.0(10.0)</td>
<td>11.3(5.9)</td>
<td>28(11)</td>
</tr>
<tr>
<td>Shallow in UVT</td>
<td>64.0(3.0)</td>
<td>48.3(4.4)</td>
<td>8.5(2.9)</td>
<td>18(6.3)</td>
</tr>
<tr>
<td>Deep in UVO</td>
<td>65.5(2.4)</td>
<td>63.8(2.4)</td>
<td>9.0(0.7)</td>
<td>14(1.1)</td>
</tr>
<tr>
<td>Shallow in UVO*</td>
<td>79.3(4.1)</td>
<td>73.3(3.4)</td>
<td>3.0(1.5)</td>
<td>3.9(2.3)</td>
</tr>
<tr>
<td><strong>Experiment III</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deep in UVT</td>
<td>n/a</td>
<td>27.0(3.4)</td>
<td>5.0(2.0)</td>
<td>19(7.8)</td>
</tr>
<tr>
<td>Shallow in UVT</td>
<td>n/a</td>
<td>24.3(3.4)</td>
<td>3.3(1.7)</td>
<td>15(8.5)</td>
</tr>
<tr>
<td>Deep in UVO</td>
<td>n/a</td>
<td>40.3(8.8)</td>
<td>16.8(3.3)</td>
<td>42(2.5)</td>
</tr>
<tr>
<td>Shallow in UVO</td>
<td>n/a</td>
<td>38.3(5.0)</td>
<td>6.5(1.3)</td>
<td>18(4.4)</td>
</tr>
</tbody>
</table>
Fig. 5.3A-C. Mean (± SE) total survival and total recruitment of *Pocillopora damicornis* larvae in Experiments I (A), II(B), and III(C). (A) Larvae were obtained from UVT and UVO acclimated adults (Exp. I), (B) and (C) larvae originated from shallow (<0.5 m) and deep (2 to 3 m) (Exp. II and III), and were exposed to either UVT or UVO conditions in the larval recruitment chambers placed at 0.5 m. Sample sizes were four chambers per treatment except in (B), n = 3 for deep in UVT and shallow in UVO. Shal = shallow.

UVT = UV-transparent. UVO = UV-Opaque.
A. Exp I

B. Exp II

C. Exp III

Total Survival

Total Recruitment

No. of larvae

UVO in UVT  UVT in UVT  UVO in UVO  UVT in UVO

Deep in UVT  Shal in UVT  Deep in UVO  Shal in UVO

No. of larvae

Deep in UVT  Shal in UVT  Deep in UVO  Shal in UVO
Table 5.2. Results of two-way ANOVA for survival, recruitment, and location of recruitment of *Pocillopora damicornis* larvae from adult colonies originating from UVT or UVO incubation (Experiment I) and shallow or deep (Experiments II and III), exposed to either ambient UVR or no UVR. Results based upon the SAS Institute's general linear model procedure. MS = mean square. \( P \)-values less than 0.05 highlighted in bold type. The dependent variable “% of recruits in sun” was arcsine transformed.

<table>
<thead>
<tr>
<th>Dependent Variable</th>
<th>Origin of Larvae</th>
<th>UVR</th>
<th>Origin X UVR</th>
<th>Error</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experiment I</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Survival</td>
<td>( df )</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>MS</td>
<td>72.25</td>
<td>380.3</td>
<td>56.25</td>
<td>92.58</td>
</tr>
<tr>
<td></td>
<td>( F )</td>
<td>0.78</td>
<td>4.11</td>
<td>0.61</td>
<td></td>
</tr>
<tr>
<td></td>
<td>( P &lt; )</td>
<td>0.394</td>
<td>0.066</td>
<td>0.451</td>
<td></td>
</tr>
<tr>
<td>Recruitment</td>
<td>( df )</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>MS</td>
<td>95.06</td>
<td>1106</td>
<td>14.06</td>
<td>98.98</td>
</tr>
<tr>
<td></td>
<td>( F )</td>
<td>0.96</td>
<td>11.17</td>
<td>0.14</td>
<td></td>
</tr>
<tr>
<td></td>
<td>( P &lt; )</td>
<td>0.346</td>
<td>0.0059</td>
<td>0.713</td>
<td></td>
</tr>
<tr>
<td>% of recruits in sun</td>
<td>( df )</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>MS</td>
<td>0.0136</td>
<td>0.2452</td>
<td>0.0255</td>
<td>0.1023</td>
</tr>
<tr>
<td></td>
<td>( F )</td>
<td>0.13</td>
<td>2.40</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td></td>
<td>( P &lt; )</td>
<td>0.723</td>
<td>0.148</td>
<td>0.626</td>
<td></td>
</tr>
<tr>
<td><strong>Experiment II</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Survival</td>
<td>( df )</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>MS</td>
<td>57.17</td>
<td>106.9</td>
<td>325.9</td>
<td>36.63</td>
</tr>
<tr>
<td></td>
<td>( F )</td>
<td>1.56</td>
<td>2.92</td>
<td>8.90</td>
<td></td>
</tr>
<tr>
<td></td>
<td>( P &lt; )</td>
<td>0.240</td>
<td>0.118</td>
<td>0.014</td>
<td></td>
</tr>
<tr>
<td>Recruitment</td>
<td>( df )</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>MS</td>
<td>242.9</td>
<td>1961</td>
<td>4.667</td>
<td>97.42</td>
</tr>
<tr>
<td></td>
<td>( F )</td>
<td>2.49</td>
<td>20.1</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td>( P &lt; )</td>
<td>0.145</td>
<td>0.0012</td>
<td>0.8312</td>
<td></td>
</tr>
<tr>
<td><strong>Experiment III</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recruitment</td>
<td>( df )</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>MS</td>
<td>22.56</td>
<td>742.6</td>
<td>0.563</td>
<td>125.4</td>
</tr>
<tr>
<td></td>
<td>( F )</td>
<td>0.18</td>
<td>5.92</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>( P &lt; )</td>
<td>0.679</td>
<td>0.032</td>
<td>0.948</td>
<td></td>
</tr>
<tr>
<td>% of recruits in sun</td>
<td>( df )</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>MS</td>
<td>0.6323</td>
<td>0.5669</td>
<td>0.1052</td>
<td>0.1332</td>
</tr>
<tr>
<td></td>
<td>( F )</td>
<td>4.74</td>
<td>4.25</td>
<td>0.79</td>
<td></td>
</tr>
<tr>
<td></td>
<td>( P &lt; )</td>
<td>0.050</td>
<td>0.062</td>
<td>0.392</td>
<td></td>
</tr>
</tbody>
</table>
**HPLC analysis of MAAs**

The larvae of *P. damicornis* had the same suite of MAAs present in their tissues as the adult corals. There were six MAAs identified in the extractions: mycosporine-glycine, shinorine, porphyra-334, palythine, asterina-330, palythene, and one unknown compound. Adult corals incubated in the presence of UVR had two-fold higher concentrations of identified MAAs than adults kept in the absence of UVR (p<0.015) (Table 5.3). A significant difference in total identified MAA concentration was not detected in the adults from <0.5 m and those from 2 to 3 m (p<0.253) (Table 5.3), although the mean concentration of the shallow adults was double that of the deep adults.

Larvae originating from shallow corals had significantly higher amounts of identified MAAs than larvae from depth (p<0.0058) (Table 5.3). Unfortunately the extractions of the UVO and UVT larvae were too dilute because less material was available for extraction, and measurements were too close to detection limits to quantify.
Table 5.3. Results of HPLC analysis for larvae and adult *Pocillopora damicornis*, with accompanying two-sample t-tests for differences between origins. Variance was assumed to be equal except where marked (*). UVT = UV-transparent. UVO = UV-opaque. Values are mean (standard error) MAAs (nmol g\(^{-1}\) skeleton or nmol larva\(^{-1}\)).

<table>
<thead>
<tr>
<th>Adult Origin</th>
<th>Total identified MAAs (nmol g(^{-1}) or larva(^{-1}))</th>
<th>Two-sample t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>UVT</td>
<td>163.8(15.3)</td>
<td>t-value 4.09</td>
</tr>
<tr>
<td>UVO</td>
<td>80.2(13.6)</td>
<td>p-value &lt;0.015</td>
</tr>
<tr>
<td>Shallow</td>
<td>240.0(76.5)</td>
<td>t-value* 1.57</td>
</tr>
<tr>
<td>Deep</td>
<td>118.5(13.0)</td>
<td>p-value* &lt;0.253</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Larval Origin</th>
<th>Total identified MAAs (nmol larva(^{-1}))</th>
<th>Two-sample t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shallow</td>
<td>0.0501(0.0099)</td>
<td>t-value* 2.81</td>
</tr>
<tr>
<td>Deep</td>
<td>0.0207(0.0032)</td>
<td>p-value* &lt;0.031</td>
</tr>
</tbody>
</table>
DISCUSSION

The results of all three experiments show that UVR had a negative effect on the recruitment of *Pocillopora damicornis* larvae. The presence of UVR resulted in a 26 to 35% reduction in larval recruitment. There was no effect of larval origin as hypothesized. Larvae from adults in the shallow habitat or from the adults incubated in UVR did not have higher survivorship or recruitment in the UVT treatment than those from deep habitat or incubated in the absence of UVR. This seemed surprising considering that larvae from shallow colonies had significantly greater concentrations of MAAs than the larvae from deep colonies. These results indicate that MAAs may not perform a photoprotective role in this species, but that larvae may avoid recruiting to the substrate when UVR is present.

*The role of mycosporine-like amino acids*

When the MAA concentrations for *Agaricia agaricites* planulae originating at 3 m reported by Gleason and Wellington (1995) are expressed in nmol larva\(^{-1}\) (using their reported value of 25 \(\mu g\) protein larva\(^{-1}\)), their values are two orders of magnitude higher than those found here. They extracted 25 planulae in 500 \(\mu l\) and detected chromatographic peaks up to 0.03 absorbance units. In this study, extracting 10 planulae in 100 \(\mu l\) of methanol resulted in absorbance values of all peaks below detection limits. It is possible that *Pocillopora damicornis* may not supply the brooded larvae with MAAs at the level found in *Agaricia agaricites*, but caution must be used regarding this because an unknown compound from *P. damicornis* could not be quantified.
In addition to Gleason and Wellington (1995), other researchers have sought to provide evidence for the adaptive role of MAAs in larval ecology. Adams and Shick (1996) demonstrated that cleavage delay caused by UVR in the green sea urchin, *Strongylocentrotus droebachiensis*, was reduced in eggs containing high concentrations of MAAs. A similar study on the eggs of the sea hare, *Aplysia dactylomela*, did not clearly demonstrate the photoprotective role of MAAs (Carefoot et al. 1998). They found no effect of diet (MAA-rich or MAA-poor) on developmental rates or larval size at hatching, and results regarding hatching success were equivocal. In other studies, MAAs were found to be concentrated in the ovaries of the sea cucumber *Holothuria atra* (Bandaranayake and Des Rocher 1999) and associated with sexual maturity in the sponge *Dysidea herbacea* (Bandaranayake et al. 1997). These authors suggested that MAAs may perform roles associated with the reproductive cycle in these species rather than UVR protection.

Other recruitment studies

A study by Baker (1995) demonstrated inhibitory effects of UVR on recruitment of *Pocillopora damicornis* larvae. His study revealed higher percent recruitment of larvae in petri dishes exposed to PAR only as compared to PAR plus UV-A and PAR plus UV-A and UV-B. His study did not reveal any UVR treatment effects on larval survival, nor an effect of larval origin (larvae were obtained from adults acclimated to the UV exposures above) on survival or recruitment. These results are very similar to those of the present study.
Recently, Mundy and Babcock (1998) demonstrated that the larvae of different coral species respond differently to spectral quality and light intensity. In five of the six species tested, larvae remained in the water column as opposed to settling in certain high intensity light treatments. These observations are consistent with the results of this study. In fact, UVR could have been responsible for their findings, as the spectra reported for the filters used in their experiments allowed transmittance in the UV region.

Numerous studies report that coral larvae preferentially recruit to the vertical or underside surfaces of recruitment tiles placed at less than 5 m depth (Tomascik 1991; Maida et al. 1994; Maida et al. 1995; Babcock and Mundy 1996). A number of factors such as sedimentation, algal growth, predation by grazers, and light intensity on the horizontal surfaces, have been put forward to explain the phenomenon. So far, no one has invoked UVR to explain this pattern. Babcock and Davies (1991) specifically tested the hypothesis that this pattern could result from sedimentation rates, and found that high sedimentation affected the location of recruitment (higher sedimentation resulted in less recruits on the upper surface of tiles) but did not affect total recruitment. The present study showed a negative effect of UVR on total recruitment, but no effect on location of recruitment. The cue resulting in the decision not to settle may have overpowered any cue related to where to settle. The sedimentation effect found by Babcock and Davies may be important at a latter stage of the recruitment process than the UVR effect. Thus, high UVR followed by high sedimentation could act in sequentially to reduce recruitment rates to exposed horizontal surfaces.
Mechanism of UVR detection

Members of the Phylum Cnidaria are at the tissue level of organization and lack a centralized nervous system. Hyman (1940) notes that some medusoid stages of Class Hydrozoa and Class Scyphozoa possess ocelli: pigment-filled sensory cells that function as eyespots. Hyman states that members of the phylum lacking ocelli that respond to light must perceive light intensity via the general sensory epithelium. Coral larvae have long been recognized to be phototactic, positively so at first release and then negatively so thereafter until recruitment (Edmondson 1929; Lewis 1974). The site of perception for the apparent sensitivity to UVR observed in this study is unknown. Detection of UVR could be via an indirect process; it is not necessary to perceive UVR directly in order to develop avoidance behavior. Perhaps UVR affects the bacterial film or the chemical composition of crustose coralline algae that may be necessary to trigger coral larva metamorphosis (Morse et al. 1988). The equivocal results regarding the effects of UVR on location of recruitment in this experiment support this idea. UVR had a general, negative effect on larval recruitment.

Implications for coral reproductive ecology

This study clearly demonstrates a link between the presence of ambient UVR and coral recruitment. The results illustrate the significance of UVR as an important physical parameter that affects the ecology of P. damicornis. It has been shown that ambient UVR negatively affects adult coral growth, while increasing planulae production in P. damicornis (Jokiel and York 1982). Living in high irradiance environments may come with a cost, resulting in the evolution of adaptive mechanisms in P. damicornis.
Environmental pressures may have selected for allocation of energy toward production of planulae when UVR is particularly high, as well as for an avoidance response of high UVR at the planulae stage.
CHAPTER 6: SUMMARY

MAIN CONCLUSIONS

The main conclusions that can be drawn from this work are:

- The surface flux of UVR in Kaneohe Bay on a cloudless day during the winter is slightly more than half (≈ 66%) of that in the summer. The corresponding amount of UV-B is 55%. PAR is reduced to 76% of the summer value.

- Cloud cover decreases solar irradiance quite dramatically. A low-altitude cumulus cloud, for example, decreased PAR by 73%, UVR by 67% and UV-B by 62%. It is interesting to note that the proportion of UVR making up the total irradiance actually increases when it is cloudy due to the fact that the clouds attenuate PAR more than UVR.

- Total MAA concentration in the tissues of *Porites compressa* and *Montipora verrucosa* does not significantly mirror the seasonal change in UVR exposure. Shinorine was the only MAA significantly related to UVR levels in *P. compressa*, and palythene was the only one in *M. verrucosa*.

- Photosynthetic pigments showed the opposite relationship with solar radiation, nearly doubling in concentration during the winter in both species. In *P. compressa*, total pigments·cm⁻² surface area in summer were 66.2% of that in winter. In *M. verrucosa*, total pigments·gram⁻¹ skeletal weight in summer was 67.5% of that in winter.
• In *M. verrucosa*, the chl $\text{a}_2$ : chl a ratio increased in winter months. These results indicate that the zooxanthellae in this species may photoacclimate to lower light availability by enhancing the amount of this accessory pigment in the light-harvesting complexes. The putative photoprotective pigment, diadinoxathin, did not increase in proportion to any of the light-harvesting pigments anytime during the year. In *P. compressa*, however, there was evidence of diadinoxanthin enrichment during the summer months. These results indicate that the zooxanthellae within the two species of coral investigated in this study may have different methods of photoacclimation, which may help explain their distribution in nature. *P. compressa* tends to be concentrated at the reef crest and reef flat, whereas *M. verrucosa* has its peak distribution on the reef slope.

• Exposure to ambient UVR is the most important factor tested in determining the concentration of MAAs in the tissues of *P. compressa*. MAA concentration was either enhanced or sustained in corals exposed to ambient UVR, and declined in corals shielded from UVR.

• When UVR was experimentally eliminated from the environment of *P. compressa*, MAA concentration decreased slowly over time. In the tank experiment (Chapter 3), corals placed in the UVO treatment in low water motion (UVO LO) experienced a 29.5% loss in total MAAs over the six week experiment. Corals in the UVO treatment in high water motion (UVO HI) only experienced a corresponding 15.8% loss. In the field experiment (Chapter 4), the corals in the UVO LO treatment experienced a 36.1% loss in
total MAAs over a six-week period, and those in the UVO HI treatment, a 29.2% loss. It appears that high water motion allows for either a slower degradation rate of the molecules or for their continued production on a limited basis.

• Exposure to ambient UVR had no effect on the concentration of photosynthetic pigments in *P. compressa* in either of the experiments, flume or field.

• Ambient levels of UVR did not affect calcification rate of *P. compressa*, except in the UVT HI treatment of the flume experiment. These corals were experiencing stress from exposure to a water motion regime to which they were not acclimated, and UVR apparently acted synergistically with this stress to cause lower calcification rates than in the other treatments.

• Water motion affected the surface area specific concentration of photosynthetic pigments in *P. compressa*. First it must be noted that in the flume experiment, the water motion treatments were HI = 14.6 ± 1.4 cm s⁻¹ and LO = 3.1 ± 0.6 cm s⁻¹. In the field experiment, HI = 7.1 ± 3.1 cm s⁻¹ and LO = 1.7 ± 0.2 cm s⁻¹. Thus, direct comparison of the two experiments with regard to water motion must proceed with this disparity in mind. In the flume experiment, being exposed to water motion of 14.6 cm s⁻¹ for six weeks resulted in an average 33% increase in chl a cm⁻², whereas 3.2 cm s⁻¹ resulted in only a 2.3% increase. In the field experiment, corals placed at the point reef, where water motion averaged 7.1 cm s⁻¹, resulted in a 9.0 % increase in total pigments (chl a and chl
Corals relocated to the leeward site, with water motion of 1.4 cm s⁻¹, lost an average 21.8% of their total pigment pool. The low water motion treatment in the flume experiment and the high water motion site in the field experiment are within the “ambient” range for this species, whereas the high flume is above average, and the low field site is probably below average (although this species does naturally occur there). Thus, being moved to a water motion regime above that to which the corals were acclimated resulted in a substantial pigment gain, while being moved to lower water motion resulted in a pigment loss of similar magnitude.

- Water motion affected the calcification rate of *P. compressa*. This effect was clearly demonstrated in the field experiment, where calcification rate at the low water motion site was 11.6% lower than at the high water motion site. It must be noted that calcification rates in the flume experiment were roughly double that in the field experiment. The reason for this disparity is uncertain, but it could be due to differences in irradiance or the unidirectional flow created in the flume which is unlike the oscillatory wave-induced motion in the field. Calcification rates in the flume experiment were more dependent upon time (duration of the treatment) than any other factor; the corals grew much faster during the second three weeks than the first three weeks of the experiment. Lateral growth of tissue around the base of the corals (development of a “skirt”) was a ubiquitous observation in the flume experiment, but was rarely observed in the field experiment.

- The field experiment revealed that different colonies of *P. compressa* had the same suite of MAAs, but that the proportion and overall quantity of MAAs varied among
colonies. Branches from within a single colony had very similar proportions and concentrations of MAAs. One colony, for instance, was completely lacking a particular MAA, with every replicate from that colony lacking the MAA. Thus, it was important that the field experiment was blocked with respect to coral colony, having one representative branch from each colony in each of the treatments. These population-wide differences in MAA distribution are interesting from an ecological perspective, and may help explain the mosaic bleaching patterns observed during most bleaching events. Corals of the same species residing side by side may have different amounts and/or composition ratios of MAAs within their tissues.

- Ambient UVR decreased recruitment rate in the three field experiments performed with *Pocillopora damicornis* larvae. Ambient UVR did NOT cause higher mortality rate in these experiments.

- The origin of the larvae (shallow, deep, UVT, and UVO) affected neither the recruitment rate nor the survival of *P. damicornis* larvae. This result occurred despite the fact that shallow and UVT adults contained roughly double the amount of MAAs than their deep and UVO counterparts, and that shallow larvae contained over twice the amount of MAAs larva$^{-1}$ than the deep larvae. Results from UVT and UVO larvae were unfortunately unobtainable. The lack of an origin effect suggests that either MAAs do not perform an ecologically important role in larval ecology of this species, or that even corals from deep and those pre-treated in UVO conditions have MAA concentrations above the threshold needed for photoprotection.
THE ROLE OF MAAs IN THE CORAL-ALGAL SYMBIOSIS

The results of this study suggest that MAAs may serve a photoprotective role in the coral-algal symbiosis. The study on the variation of MAAs during an annual cycle in *Porites compressa* and *Montipora verrucosa* (Chapter 2) revealed that the concentrations of two MAAs correlate with UVR exposure, although the relationship was not as pronounced as expected. However, if one compares the relative change in UVR exposure on an annual basis and that on a depth gradient, the results of the study become more understandable. Winter UVR values are about 66% of those in summer. This change would correspond to moving from just below the surface of the water to a depth of only 1.2 m. Thus, it is nonsensical to expect the order of magnitude change in MAA concentration that is observed along a depth gradient.

Chapters 3 and 4 demonstrated that *P. compressa* responds to the presence of UVR by producing and/or accumulating MAAs. MAAs are nitrogenous compounds. If the widespread notion that zooxanthellae are nitrogen-limited is true, then these molecules may be expensive for the zooxanthellae to produce, if they are, in fact, producing them. The results of the field experiment (Chapter 4) supported this notion, as the UVT HI treatment had the highest mean concentration of MAAs at the end of the six-week experiment. The flume experiment (Chapter 3) displayed this same response during the first three weeks of the experiment. Reduction in thickness of the boundary layer could have resulted in increased uptake rates of ammonium and/or nitrate from the water column by the symbiosis, leading to higher concentrations of MAAs in the
presence of UVR. The fact that corals subjected to the UVO HI treatment continued to lose MAAs despite the increase in water motion supports the notion that corals and their zooxanthellae will not “waste” nitrogen or other resources on producing and/or accumulating MAAs in the absence of UVR.

The discontinued production of MAAs in the absence of UVR could be used as evidence that the molecules do impose a cost to the symbiosis, or it could simply indicate the absence of the cue needed to trigger their production. In support of the latter, there was no increase in growth rate and/or photosynthetic pigment concentration in *P. compressa* when UVR was removed from the environment (Chapters 3 and 4). This result indicates that the production of MAAs does not impose a large enough cost to the symbiosis to be reflected in reduced growth or photosynthetic capacity. This fact could also be used as support for the idea that MAAs are “doing their job” in protecting the symbiosis from the negative effects of ambient UVR without imposing a huge cost to the organisms.

The results of Chapter 5 suggested that MAAs may not play a crucial role in the larval ecology of *Pocillopora damicornis*, or that the amount of MAAs in all larvae is above the threshold needed for photoprotection. The origin of the larvae, which determined the concentration of MAAs per larva (confirmed for the shallow/deep larvae), did not have an effect on either larval survival or recruitment. This was surprising considering the results of Gleason and Wellington (1995), who showed increased mortality of deep, MAA-poor larvae compared to shallow, MAA-rich larvae in the presence of UVR.
In sum, the results of this study indicate that at least some of the MAAs (shinorine and palythene) are positively correlated with the presence of UVR. Also, their production ceases when UVR is not present. These findings add to body of evidence that MAAs are acting as sunscreens in the coral-algal symbiosis. Concerning photosynthetic pigments, this study suggests that further investigation into the importance of the non-light harvesting xanthophyll pigments would be most informative.

FUTURE STUDIES ON UVR AND MAAs IN CORALS

The results of Chapter 5 indicate that UVR is important to the larval ecology of Pocillopora damicornis. Ambient UVR inhibited larval recruitment, without increasing mortality. These results are important to coral reef management and rehabilitation. When trying to re-seed a debilitated reef, it is important to be aware of the different factors that can affect coral recruitment. The present study is the first to reveal that UVR is important in coral recruitment. The next step in this research would be to confirm the phenomenon for different species of coral, especially broadcasting species because fertilization and early development occur on the water’s surface where UVR is high.

The trophic accumulation of MAAs within the coral reef ecosystem is another topic that warrants immediate attention. There are many secondary metabolites produced by marine plants and invertebrates that have been shown to deter grazing by other animals (Hay et al. 1987; Paul et al. 1990; Cronin and Hay 1996; see review by Hay 1996). Red algae, in general, have been shown to be more palatable to certain fishes that browns, which has been attributed to the fact that browns are known producers of
terpenes and other distasteful chemicals (Miller and Hay 1996). Red algae have been
shown to be rich in MAAs compared to their green and brown counterparts (Sivalingam
et al. 1974; Beach et al. 1997; Banaszak et al. 1998). The hypothesis that the that
preferential consumption of red algae is partially due to their being a rich source of
MAAs needs to be tested.

It would also be instructive to conduct nutrient enrichment studies. Because
MAAs contain nitrogen, it is reasonable to believe that the production of MAAs would be
enhanced when ammonium and/or nitrate levels are elevated. The results of Chapter 3
and 4 support this hypothesis in that water motion had a positive affect on MAA
concentration. Perhaps decreasing the thickness of the diffusive boundary layer allowed
higher uptake rates of nitrogen, which led to higher concentrations of MAAs. Support for
this idea would be provided if elevated nitrogen levels had the same effect.
LITERATURE CITED


Beach KS, Borgeas HB, Nishimura NJ, Smith CM (1997) In vivo absorbance spectra and the ecophysiology of reef macroalgae. Coral Reefs 16


156
Grottoli AG, Kuffner IB (1995) Uneven bleaching within a coral colony in response to
differing levels of solar radiation. In: Gulko D and Jokiel P (eds) Ultraviolet
radiation and coral reefs, HIMB Tech. Report No. 41. Sea Grant, Honolulu, pp
115-120
radiation on aquatic ecosystems. Photochem Photobiol 53: 717-725
Harriott VJ (1998) Growth of the staghorn coral *Acropora formosa* at Houtman
Abrolhos, Western Australia. Mar Biol 132: 319-325
Biol Ecol 200: 103-134
herbivores. Ecology 68: 1581-1591
Helmuth B, Timmerman B, Sebens K (1997a) Interplay of host morphology and
symbiont microhabitat in coral aggregations. Mar Biol 130: 1-10
Helmuth BS, Sebens KP, Daniel TL (1997b) Morphological variation in coral
209: 233-259
Hodgson G (1990) Sediment and the settlement of larvae of the reef coral *Pocillopora
damicornis*. Coral Reefs 9: 41-43
Hoegh-Guldberg O (1999) Climate change, coral bleaching and the future of the world's
coral reefs.
Hoegh-Guldberg O, Smith GJ (1989) Influence of the population density of
zooxanthellae and the supply of ammonium on the biomass and metabolic

157
characteristics of the reef corals *Seriatopora hystrix* and *Stylophora pistillata*.

Mar Ecol Prog Ser 57: 173-186


Mason DS, Schafer F, Shick JM, Dunlap WC (1998) Ultraviolet radiation-absorbing mycosporine-like amino acids (MAAs) are acquired from their diet by medaka fish (Oryzias latipes) but not by SKH-1 hairless mice. Comp Biochem Physiol 120A: 587-598


Patterson MR, Sebens KP, Olson RR (1991) In situ measurements of flow effects on primary production and dark respiration in reef corals. Limnol ocean 36: 936-948


