# RESPONSES OF TWO SPECIES OF CARIBBEAN SHALLOW-WATER BRANCHING CORALS TO CHANGES IN ULTRAVIOLET RADIATION

By

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A thesis submitted in partial fulfillment of the requirements for the degree of
Doctor in Philosophy
In
Marine Sciences
UNIVERSITY OF PUERTO RICO
MAYAGÜEZ CAMPUS
2005

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# ABSTRACT

Clear coral reefs waters are highly transparent to ultraviolet radiation (UVR) and therefore, reef organisms should be adapted to tolerate present levels of UVR. However, UVR can severely damage coral tissues and overall physiology. The effects of changes in UVR on the growth, fecundity, and photosynthetic and photoprotective pigments production of the Caribbean shallow-water branching corals *Acropora cervicornis* (Lamarck 1816) and *Porites furcata* (Lamarck 1816) were studied by either reducing or enhancing normal UVR doses in two separate experiments. First, UVR was artificially depleted with Hyzod® acrylic panels and Saran® meshes. Second, different colonies were exposed to enhanced UVR by either transplanting colonies of *A. cervicornis* from deep to shallow areas, or exposing colonies of *P. furcata* to 10% increased UVR in aquariums located under UV fluorescent bulbs. Growth rates were measured using the Alizarin Red staining method. Fecundity was estimated after histological analysis. Pigments were quantified through High Performance Liquid Chromatography (HPLC) analysis. A positive correlation was found between growth and photosynthetic pigments concentration, and reduced UVR, while the concentration of UV-absorbing compounds (mycosporine-like amino acids or MAA's) was negatively correlated with reduced UVR. Severe bleaching experienced by *A. cervicornis* colonies transplanted from deep to shallow areas resulted in significantly decreased growth rates and photosynthetic pigments concentration compared to controls, although no significant changes were observed in zooxanthellae densities. This suggests that a specific targeted effect of UVR on the photosynthetic capacity of the zooxanthellae caused the bleaching. Bleached

colonies survived by significantly increasing the UVR protection with increased MAA's concentrations and a possible relocation of resources. Similarly, colonies of *P. furcata* exposed to artificially enhanced UVR significantly reduced their growth rates and photosynthetic pigments concentrations compared to controls exposed to normal UVR. A significant increase in MAA's was also found in colonies of *P. furcata* under enhanced UVR, while no differences were observed in fecundity compared to controls. While several physical factors may influence reef corals physiology, these results suggest that shallow-water corals could be significantly affected by increases in UVR resulting from the thinning of the Earth's ozone layer.

# RESUMEN

Las aguas arrecifales claras son altamente transparentes a la radiación ultravioleta (UVR) por lo que los organismos arrecifales deben estar adaptados a tolerar niveles actuales de UVR. Sin embargo, UVR puede dañar severamente los tejidos y la fisiología de los corales escleractínidos. Los efectos de cambios en UVR en el crecimiento, fecundidad, y producción de pigmentos de los corales ramificados caribeños *Acropora cervicornis* (Lamarck 1816) y *Porites furcata* (Lamarck 1816) fueron estudiados en dos experimentos separados. Primero, UVR fue reducida artificialmente con paneles de acrílico Hyzod® y mallas de Saran®. Segundo, diferentes colonias fueron expuestas a aumentos en UVR ya sea transplantando colonias de *A. cervicornis* desde áreas profundas hacia áreas llanas, ó exponiendo colonias de *P. furcata* a un aumento de 10% en UVR en acuarios localizados debajo de lámparas fluorescentes de luz ultravioleta. Las tasas de crecimiento fueron medidas usando Alizarina Roja. La fecundidad fue estimada con análisis histológico. Los pigmentos fueron cuantificados utilizando análisis de Cromatografía Líquida de Alta Precisión (HPLC). El crecimiento y la concentración de pigmentos fotosintéticos correlacionaron positivamente con UVR reducida, mientras que la concentración de pigmentos absorbentes de UVR (MAA's) correlacionó negativamente con UVR reducida. Un blanqueamiento severo en las colonias de *A. cervicornis* transplantadas de aguas profundas a aguas llanas resultó en una disminución significativa en el crecimiento y en la concentración de pigmentos fotosintéticos, sin ocurrir cambios significativos en la densidad de zooxantelas, comparadas con los controles, sugiriendo un efecto específico de UVR en la capacidad fotosintética de las

zooxantelas. Las colonias blanqueadas sobrevivieron aumentando significativamente los niveles de MAA's y una posible relocalización de recursos. Igualmente, colonias de *P. furcata* expuestas artificialmente a aumentos en UVR redujeron significativamente su crecimiento y concentraciones de pigmentos fotosintéticos, y aumentaron significativamente las concentraciones de MAA's en comparación con controles expuestos a niveles normales de UVR, pero no se observaron diferencias significativas en la fecundidad. Mientras que otros factores físicos pueden influenciar la fisiología de los corales escleractínidos, estos resultados sugieren que corales de aguas llanas pueden ser afectados significativamente por aumentos en UVR a consecuencia del adelgazamiento de la capa de ozono terrestre.

To my family, who have always believe in me, and

to the people from whom I never received a NO for an answer whenever I needed their help, my friends: the workers at Magueyes.

To all of you my most sincere thanks, respect and admiration.

# ACKNOWLEDGMENTS

My most sincere thanks to my Graduate Committee: Dr. Roy A. Armstrong, Dr. Jorge Corredor, Dr. Ernesto Weil and Dr. Fernando Gilbes, for all their help and advice during all these years. Their comments and corrections greatly improved the final version of this document.

I would like to acknowledge all the help provided by the personnel of the Fisheries Investigations Laboratory of the Puerto Rico Commonwealth, Department of Natural and Environmental Resources, especially the director, Aida Rosario, Miguel Figuerola and Wilfredo Torres for the use of the facilities and equipment of the histology laboratory. I will also always appreciate the help provided by some of the students of the Department of Marine Sciences, especially Nilda Jiménez for her help during the field work of the UV-Exclusion Experiment, and Rocío García and Oswaldo Cárdenas who patiently taught me the use and tricks of the HPLC equipment at the Chemical Oceanography Laboratory at Magueyes Island. I am grateful for the opportunity provided by the Department of Marine Sciences of the University of Puerto Rico at Mayagüez and it personnel, especially Godoberto López, Milton Carlo and Marcos Rosado who provided field work help during the setup and end of both experiments. Mr. Neftalí Figueroa and Ángel Camacho (Negrito) provided small boats whenever these were needed and no questions asked. The maintenance personnel at Magueyes aided during the construction of the UV-Supplementation Experiment.

I would like to thank my wife, Gloryvee Carrero for her outstanding courage, patience, help, and love. I also want to thank my family who have always believed in me

and gave me support whenever I needed it. Thanks to the ruler of this world and the world to come, Jesus Christ.

This work was supported by several fellowships and assistantships. This project was financed in part by NASA grant NCCW-0088. The Alliance for Graduate Education and the Professoriate (AGEP) and the Department of Biology of the University of Puerto Rico at Mayagüez provided graduate assistantships during several years. Part of this project was supported by a fellowship from the National Science Foundation's program Puerto Rico Graduate Teaching Fellows in K-12 Education. Thanks to the National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Habitat Conservation Division's Caribbean Field Office where I have been and continue working as a contractor during the past year, especially the regional director Dr. Miles Croom and the director of the office Dr. Lisamarie Carrubba.

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# INTRODUCTION

The Sun is responsible for the development and continued existence of life on Earth. The deleterious effects of sunlight on biological systems are due almost entirely to radiation within the ultraviolet spectrum of the Sun's emission. The depletion of the Earth's ozone layer and the consequent increases in ultraviolet radiation (UVR; 280-400nm) levels reaching the Earth has captured the attention of many scientists and the public during the last decades. Recent global assessments of the depletion of stratospheric ozone have shown increases of unweighted UV-B radiation (280-320nm) of up to 130% in the Antarctic, 22% in the Artic, 4-7% at temperate latitudes, and less variation at the tropics (Madronich et al., 1995). Yet, small decreases in the stratospheric ozone concentration may produce large UVR increments (Madronich et al., 1998).

Solar UVR is detrimental to many forms of life. High levels of UV reach the surface of the earth's tropics due to the thinning of the earth's protective ozone layer and the lower zenith angles near the equator (Baker et al., 1980). Current predictions suggest that higher irradiances of UV-B radiation will affect the marine environment for at least the next 25 years, if not longer (Madronich et al., 1998). Ozone depletion has particular concern at any latitude, which results in an increase in damaging UV-B wavelengths without a proportional increase in longer UV-A (320-400nm) and blue wavelengths involved in photoreactivation and photorepair (Smith, 1989). The harmful effects of UVR may involve damage to DNA and proteins (Peak and Peak, 1990), oxidation of membrane lipids, and photooxidation of chlorophyll or damage to photosystem II (Halliwell and Gutteridge, 1999). Middle UV radiation (UV-B) disrupts many

photosynthetic processes including the electron transport system, photosystem II reaction centers, and pigment stability (Karsten et al., 1998). Both UV-A and UV-B reduce algal growth rates (Bothwell et al., 1994). Current UV-B levels affect the balance between primary producers and consumers in shallow water benthic communities. Elevated levels of UV-B could augment these impacts by increasing depths at which benthic grazer communities are exposed to deleterious UV-B irradiance without a corresponding increase in the UV-A:PAR ratio.

Coral reefs require clear waters as one of the most important characteristics of their environment for optimum development. The combined optical properties of the various features that comprise ecosystems such as coral reefs have a first-order effect on the intensity and spectral composition of the adjacent light field (Ackleson, 2003). Clear ocean water, such as those found over many coral reefs areas, is notably transparent to UVR (Jerlov, 1950; Smith and Baker, 1979). Penetration of UVR, particularly UV-B depends strongly on the optical properties of the water itself, and the dissolved and suspended organic materials (Calkins, 1982).

The notable success of corals and other cnidarians containing symbiotic algae might be attributed to their ability to tolerate UVR. The plant and animal fractions might not be capable of independently offsetting the high metabolic cost of UVR encountered on shallow reefs, but can do so as a symbiotic unit (Jokiel, 1980). This suggests that corals and other shallow-water sessile zooxanthellate organisms have evolved mechanisms to protect their tissues from the damaging effects of ultraviolet radiation. Indeed, one such photoprotective mechanism exists in the form of mycosporine-like amino acid compounds (MAA's) (Shibata, 1969).

The possible effects of UVR on reef-building corals have received widespread attention in the Pacific during the last decades (e.g., Siebeck, 1981; Dunlap et al., 1988; Drollet et al., 1993; Kinzie, 1993; Masuda et al., 1993; Krupp and Blanck, 1995; Lewis, 1995; Lesser and Lewis, 1996; D'Croz et al., 2001; Kuffner, 2001a, b). Comparatively less literature exists on the response of Caribbean corals to this factor (Gleason, 1993; Gleason and Wellington, 1993, 1995; Muszynski, 1997; Muszynski et al., 1998; Corredor et al., 2000).

So far, no study has quantified the effects of enhanced ultraviolet radiation on shallow-water Caribbean branching corals. These organisms have similar UV-protecting compounds as those in the Pacific (Banaszak et al., 1998). There are, however, some reports on the effects of UVR (i.e., inhibition of photosynthesis; changes in the zooxanthellae concentration; lower algal mitotic indices; lower linear skeletal extensions; among others) on Caribbean massive coral species (Gleason, 1993; Gleason and Wellington, 1995; Lesser et al., 2000).

This work explores the possible detrimental effects that increases in UVR (mediated by a reduction in the ozone layer) may cause to shallow-water corals and the possible biological implications (i.e., decreased coral linear extension rates and calcification rates; decrease in the energy and resources available for defense; increase in UV-induced coral bleaching; among others). Some ecological implications of enhanced UVR levels may include: reduction in the competitive ability of corals; changes in the species zonation with the more UV-adapted species dominating greater depth ranges; and increased mutation rates.

The objectives of this study were:

1. To characterize the annual UV irradiance and dose incident on a shallow-water back-reef area in La Parguera, Puerto Rico.
2. To assess the effects of changes in UVR on: a) growth rates; b) fecundity; and c) production of photosynthetic and UV-absorbing compounds in two shallow-water coral species, the staghorn coral *Acropora cervicornis* (Lamarck 1816) and the branched finger coral *Porites furcata* (Lamarck 1816).

With these objectives in mind, three different hypotheses were tested during the present study:

A. The first null hypothesis ($H_{0,1}$) establishes that there are no significant changes in the growth rates of both species with changes in UVR (increase or decrease). The alternate hypothesis ($H_{1,1}$) states that significant changes in growth rates will occur after exposing the coral colonies to enhanced or reduced UV levels.
B. The second null hypothesis ($H_{0,2}$) states that reproductive effort (measured as the number of eggs/bundle in the case of the broadcasting species *A. cervicornis*; and number of released larvae in the case of the brooding species *P. furcata*) is not affected by changes in UVR. The alternate hypothesis ($H_{1,2}$) states that a significant reduction in the fecundity will be observed under enhanced UVR and an increase will occur under reduced UVR.
C. The third null hypothesis ($H_{0,3}$) states that there are no significant changes in the concentration of MAA's with an increase in the UV levels. The alternate hypothesis ($H_{1,3}$) states that there will be an increase in the concentration of MAA's with

enhanced UVR and, that this will decrease with a reduction in the UVR levels reaching the colonies.

D. The fourth null hypothesis ($H_{0,4}$) states there are no significant changes in the concentration of photosynthetic pigments and zooxanthellae densities with an increase in the UV levels. The alternate hypothesis ($H_{1,4}$) states that there will be a reduction in the concentration of photosynthetic pigments and zooxanthellae densities with enhanced UVR and, an increase with reduced UVR levels reaching the colonies.

# PREVIOUS WORKS

## Penetration of Radiation in the Ocean

Strictly speaking, irradiance is the flux of light energy (radiance) incident on an infinitesimally small element of surface containing the point under consideration divided by the area of that element (Jerlov, 1951; Kirk, 1994). This flux, with units of quanta $m^{-2}$ $s^{-1}$ or watts $m^{-2}$, contains information about the integrated area distribution of radiant energy.

Light intensity generally decreases with depth in accordance with Beer's Law (Kirk, 1994). In optically shallow-water, where depth is much less than the potential for light to penetrate, a large fraction of the subsurface light reaches the ocean floor, where portions of the energy are absorbed, reflected back into the water column, or re-emitted as fluorescence. Depending on water depth and the benthic optical properties, light intensity might decrease rapidly, remain constant throughout the water column, or even increase with depth (Ackleson, 2003).

The underwater light field is modified by the angle of the incident light, the absorption and scattering of light by dissolved and particulate materials in the water, and by water molecules themselves (Kirk, 1994). The relative spectral contribution is a function of the optical properties of water, dissolved materials, and particulate content (Jerlov, 1968). The angular distribution of downwelling incident light on reef corals is controlled by the solar azimuth, reflection, refraction, scattering, and shading (Falkowski et al., 1990).

The direction of maximum sunlight intensity becomes less sensitive to Sun direction and moves toward the vertical with increases in water depth. Hence, corals growing at depth experience a more uniform directional field, with most of the light coming from the vertical (Falkowski et al., 1990). Due to the fact that coral reefs require clear water conditions to successfully develop, the spectral nature of the light field in reef waters most likely resembles that of an oligotrophic ocean, because humic substances and free-living phytoplankton are generally sparse in reef waters.

Clear reef waters are notably transparent not only to the Photosynthetically Active Radiation (PAR; 400-700nm), but also to solar UV radiation (Jerlov, 1950; Smith and Baker, 1979; Jokiel and York, 1982). In fact, as early as in the middle of the past century, Jerolv's work suggested that UV must be a significant biological factor to depths of 20m in clear oceanic waters (Jerlov, 1950, 1968). These are typical depths where platform and shelf edge reefs fully develop in the Caribbean (Goreau and Goreau, 1973).

**Interaction of Ultraviolet Radiation and the Ozone Layer**

Ozone ($O_3$), a gas that comprises approximately one molecule out of every 2,000,000 in the atmosphere, is created by the dissociation of oxygen ($O_2$) by short wavelength ($\lambda < 242$nm) UVR in the stratosphere at altitudes between 25-100km. Absorption of UVR up to 320nm converts the $O_3$ back to $O_2$ and O. This is responsible for preventing radiation of less than about 290nm from reaching the Earth's surface (Chapman, 1930).

Molina and Rowland (1974) first warned that chlorofluorocarbons (CFC's) and other gases released by human activities could alter the natural balance of creative and

destructive processes and lead to ozone depletion in the stratosphere. A decrease in the Earth's protective ozone layer and a concomitant increase in solar UVR penetration will therefore, increase both the amount and the spectral quality of UVR reaching the earth's surface (Calkins, 1982). In fact, the thickness of the ozone layer in the upper stratosphere has decreased progressively exceeding 50% of the pre-ozone hole concentrations at some locations (Farman, et al., 1986) leading to an increase in the UVR reaching the Earth's surface. Increasing $CO_2$ concentrations would result in warming of the troposphere and simultaneous cooling of the stratosphere, which favors further ozone destruction (Häder, et al., 2003).

For convenience, UVR is split into four bands (Calkins, 1982; Cockell and Knowland, 1999). Vacuum UV is radiation of wavelengths less than 200nm. UV-C radiation occupies the region between 200-280nm. None of the above reaches the surface of the Earth because of atmospheric Rayleigh scattering and ozone absorption. UV-B radiation is often defined as 280-320nm. On Earth, most of the UV-B is attenuated by the ozone column that absorbs strongly in the Hartley region (200-300nm) and weakly in the Huggins band (315-400nm). Finally, UV-A radiation (320-400nm) reaches the surface relatively unattenuated and is still less energetic than UV-B radiation. From a biological point of view, UV-B is by far the most significant part of the terrestrial UVR and its levels reaching the surface of the Earth are largely controlled by ozone (Cockell and Knowland, 1999).

Measurements under the Antarctic ozone hole have shown that whereas UV-B becomes a more significant contributor to photoinhibition, UV-A remains important (Bothwell et al., 1994). The action spectra for the UVR inhibition of phytoplankton

photosynthesis shows that per photon, shorter wavelengths (UV-B) are more disruptive than longer wavelengths (UV-A). Nevertheless, higher photon flux in UV-A usually produces the majority of the water column inhibition of photosynthesis (Bothwell et al., 1994). Some UVR effects are: DNA damage in most organisms, inhibition of photosynthetic primary productivity in both micro-organisms and higher plants, inhibition of nitrogenase activity, and reduction in microbial motility (Cockell and Knowland, 1999).

**Relationship between Global Climate Change and Growth and Photosynthesis in Reef Corals**

Light quality (i.e., sunlight hours are more important than daylight hours) is probably the most important factor influencing the rate at which $CaCO_3$ is deposited in the skeleton of a hermatypic coral (Gladfelter, 1984). For instance, it has been shown that in high light, the light-enhanced calcification rate of *A. cervicornis* is 2.8-4.0 times the dark rate (Shinn, 1966; Chalker and Taylor, 1975). This is highly influenced by the photosynthetic processes of their symbiotic algae (Muscatine and Cernichiari, 1969; Davies, 1991).

Ratios of calcification to photosynthesis appear to be affected by the ratio of alkalinity to acidity, which controls how efficiently the protons from calcification convert bicarbonate to carbon dioxide (McConnaughey, 1989, 1994; McConnaughey et al., 2000). Algae and corals use mainly bicarbonate. The seawater pool of bicarbonate and metabolically generated carbon dioxide are the primary sources of inorganic carbon for photosynthesis by zooxanthellae (Muscatine, 1990; Muscatine et al., 1981).

Vandermeulen et al. (1972) demonstrated that it was photosynthesis by the zooxanthellae which had a direct effect on enhancement of calcification in an experiment where the inhibition of photosynthesis reduced the calcification rates even in the presence of light. Calcification and photosynthesis become coupled through the reactions:

Calcification:

$$Ca^{+2} + CO_2 + H_2O = CaCO_3 + 2H^+ \quad (1)$$

Bicarbonate conversion:

$$2H^+ + 2HCO_3^- = 2CO_2 + 2H_2O \quad (2)$$

Photosynthesis:

$$CO_2 + H_2O = \text{“}CH_2O\text{”} + O_2 \quad (3)$$

Net:

$$Ca^{+2} + 2HCO_3^- = CaCO_3 + \text{“}CH_2O\text{”} + O_2 \quad (4)$$

Alkaline and acidic conditions favor reactions 1 & 2, respectively, and most calcareous organisms develop recognizable, highly alkaline calcareous zones that contrast with the noncalcareous, absorptive regions in which $HCO_3^-$ assimilation occurs. Furthermore, calcifiers tend to isolate their calcification sites, as beneath the aboral epithelium of corals, and then raise the aragonite saturation state ($\Omega_{arag}$) locally through ion transport; pH often exceeds 10 at the calcification sites. Calcification appears quite capable of stimulating photosynthesis, but this process is not metabolically “free”. Photosynthesis can also stimulate calcification. Photosynthesis increases ambient $\Omega_{arag}$, but this only slightly stimulates biological calcification (McConnaughey et al., 2000). More importantly, photosynthesis increases the alkalinity:acidity ratio, which reduces how efficiently calcification generates $CO_2$. More calcification is therefore needed to

obtain a particular photosynthetic benefit. Fleshy algae can thereby stimulate calcification in nearby corals. Coral calcification likewise counteracts $CO_2$ depletion and may stimulate photosynthesis in nearby algae.

It has been suggested (Smith and Buddemeier, 1992), and recently demonstrated (Gattuso et al., 1999; Kleypas et al., 1999; Marubini et al., 2003), that an increase in the $CO_2$ partial pressure ($pCO_2$) has a negative effect on coral and reef community calcification as a result of a decrease in $\Omega_{arag}$. For example, when a coral reef mesocosm was exposed to increased $CO_2$ partial pressure by Leclercq et al. (2002), the rate of net community calcification decreased as a function of increasing $pCO_2$ and decreasing aragonite saturation state. This re-emphasizes the predictions that reef calcification is likely to decrease during the next century (Kleypas et al., 2001). Recent work by McNeil et al. (2005) suggests that annual coral reef calcification may increase with future ocean warming. Nonetheless, their work is based on previous studies performed using only a single Indo-Pacific species and certainly more similar studies with multiple species are needed to have a better understanding on future coral reef responses.

Most of the previous works relate coral calcification and photosynthesis to global climate change factors such as $CO_2$ increases and a decrease in the $\Omega_{arag}$. However, effects of other important factors (e.g. UVR increases), in coral calcification and the reaction of their symbiotic zooxanthellae remains largely unknown.

**UVR effects on reef corals reproduction**

Reef corals may reproduce sexually or asexually. Sexually, reef corals can be either broadcast spawners (e.g. *Acropora cervicornis, Montastraea faveolata*) or brooders

(e.g., *Porites furcata, Mycetophyllia* spp.) (see reviews by Fadlallah, 1983; Szmant, 1986; Richmond and Hunter, 1990; and Soong, 1991). Corals can also reproduce asexually, with fragmentation as the most common form of asexual reproduction (Bak and Criens, 1981; Bothwell, 1981; Harrison and Wallace, 1990). Most of the world's scleractinian coral species reproduce sexually by spawning their eggs and sperm into the water column where they eventually fuse (Soong, 1991).

The effects of environmental factors on coral reproduction have been indirectly studied (i.e., by studying the effects of bleaching on reproductive aspects) in several species of cnidarians including hard (Szmant and Gassman, 1990; Omori et al., 2001) and soft (Michalek-Warner and Willis, 2001a,b) corals. Only a few studies have reported direct effects of UVR on reproductive characteristics such as larval survivorship (Gleason and Wellington, 1995; Gulko, 1995) and settlement (Baker, 1995; Kuffner, 2001b). Only Gleason and Wellington (1995) have studied the effects of UVR on the reproduction of a Caribbean coral species. The authors found a significant reduction in the planula larvae survivorship of *Agaricia agaricites* when exposed to shallower depths, and hence, higher UVR levels. So far, no study has investigated the effects of a direct increase in the UVR daily doses on coral fecundity (i.e. number of eggs or planulae produced per polyp).

**Protection of marine organisms against UVR**

Strategies of UVR mitigation in marine organisms include: avoidance (lifestyles that completely avoid solar radiation), protection (i.e., quenching of reactive oxygen species by carotenoids, mycosporine-like amino acids or MAA's, flavonoids), and repair (i.e., photoreactivation, synthesis of proteins and lipids) (Siebeck, 1988; Cockell and

Knowland, 1999). Behavioral adaptations such as avoidance are not available to sessile organisms, specifically corals that need to be exposed to the sun for their photosynthetic zooxanthellae symbionts (Kuffner et al., 1995). Damage from enhanced UVR occurs when the photoprotective defenses of the coral are exceeded.

In reef corals, high levels of solar radiation (including UVR) can increase coral mortality, inhibit skeletal growth, decrease carbon fixation and reduce photosynthetic pigment concentration (Coles and Jokiel, 1978; Jokiel, 1980; Jokiel and York, 1982, 1984). Some protection and repair mechanisms found in reef corals are: the xanthophyll cycle in photosynthesis (Ambarsari et al., 1997; Brown et al., 1999), screening pigment production (Dykens and Shick, 1984; Banaszak and Trench, 1995a, b), β-carotene production (Ambarsari et al., 1997), synthesis of antioxidants (Lesser, 1996, 1997), enzymes that reduce photooxidative stress (Gröniger et al., 1999; Häder et al., 2003), host pigmentation (Salih et al., 2000; Dove et al., 2001; Mazel et al., 2003), DNA repair (Lesser and Barry, 2003), and behavioral responses such as coral tissue retraction (Yonge, 1940).

**Mycosporine-like Amino Acids**

Mycosporine-like Amino Acids (MAA's) are water-soluble, low molecular weight substances, derivatives of cyclohexenone or cyclohexenimine, with an absorption maximum in the range between 307-360nm. The elegance of the MAA's UV-absorbance properties lies in the modulation of the peak absorbance of a basic cyclohexanone or cyclohexenimine core structure (Cockell and Knowland, 1999). Most of the UV-B-screening MAA's use a cyclohexanone structure, whereas the UV-A-screening

compounds use a cyclohexenimine core structure, presumably because the non-bonding nitrogen electrons cause a greater bathochromic shift towards the UV-A region. The subsequent incorporation of the various amino acidic or imino-alcohol groups results in the diversity of MAAs found in nature. By synthesizing a range of MAA's, organisms might be able to screen broadly in the UV-A and B range.

The MAA's are derived from early stages of the shikimic acid pathway via 3-dehydroquinic acid and 4-deoxygadusol (4-DG) (Favre-Bonvin et al., 1976, 1987; Dunlap et al., 1998; Shick et al., 1999). The Shikimate pathway is the origin to not only MAA's but also other aromatic amino acids, plastoquinones, vitamins E and K, and many other compounds found in photosynthetic microorganisms and higher plants (Bentley, 1990). The shikimate acid pathway is not found in animals even though early studies on the effects of UVR on corals assumed that it was the host tissue that produced these compounds (Jokiel and York, 1982).

Proteins and DNA are the main targets of UV-B (Häder et al., 2003); hence, UVR screening must have evolved early in the organismal history of the Earth. Some of the first screening pigments may have evolved in cyanobacteria during the Precambrian allowing the colonization of exposed, shallow-water and terrestrial habitats (Cockell and Knowland, 1999). In fact, the evolution of MAA's as specific UV-screens may represent an early innovation in dealing with Archean UV-B flux. This was further confirmed by the presence of these compounds among numerous photosynthetic organisms around the globe (Karentz et al., 1991; Banaszak et al., 1998; Karsten et al., 1998). Their ubiquitous presence across a large taxonomic and geographical range is evidence of not only their early phylogenetic innovation, but potentially also of their importance as natural UV-

screening compounds. Simpler MAA's such as mycosporine-glycine specifically absorb in the UV-B. It has been suggested that later, as oxygen levels increased, UV-A screening MAA's became important since many of the effects of UV-A are mediated through oxygen free radicals (García-Pichel, 1994) and thus, the contribution of UV-A as a damaging agent in the biosphere would have increased.

There are approximately 20 MAA's described in marine organisms (see reviews by Cockell and Knowland, 1999, and Carreto et al., 2005). The chemical structure of some of the most commonly found MAA's are shown in Figure 1. Mycosporines were first identified in fungi as having a role in UV-induced sporulation (Leach, 1965; Favre-Bonvin et al., 1976). Their relatives, the mycosporine-like amino acids, have since been found in cyanobacteria (Shibata, 1969; Karentz et al., 1991; García-Pichel and Castenholz, 1993; García-Pichel et al., 1993), red algae (Takano et al., 1979; Karentz et al., 1991; Karsten et al., 2000), other free-living algae (Karsten et al., 1998), diatoms (Moisan and Mitchell, 2001), dinoflagellates (Balch and Haxo, 1984; Carreto et al., 1990), lichens (Karentz et al., 1991), gorgonians (Michalek-Wagner, 2001), corals and their associated biota (Shibata, 1969; Dunlap and Chalker, 1986; Dunlap et al., 1986; Gleason, 1993; among others), as well as many other marine organisms such as other cnidarians (Takano et al., 1978a,b; Scelfo, 1988; Stochaj et al., 1994; Banaszak and Trench, 1995a, b), sponges (Nakamura et al., 1982), brine shrimp (Grant et al., 1985), sea urchins (Carroll and Shick, 1996; Adams and Shick 1996, 2001; Adams et al., 2001), starfish (Nakamura et al., 1982), holothurids (Shick et al., 1992), clams (Ishikura et al., 1997), ascidians (Dionisio-Sese et al., 1997), fish (Karentz et al., 1991), and fish eggs (Chioccara et al., 1980). In organisms lacking photosynthetic symbionts, the MAA's are

believed to be acquired through their respective diets. Tables 1 and 2 show a summary of MAA's found in reef corals of the Indo-Pacific and Caribbean, respectively. Large differences can be noticed in the number of coral species studied as well as the related scientific works published for both provinces. So far, there are no publications on the effects of UVR on the species used in the present work.

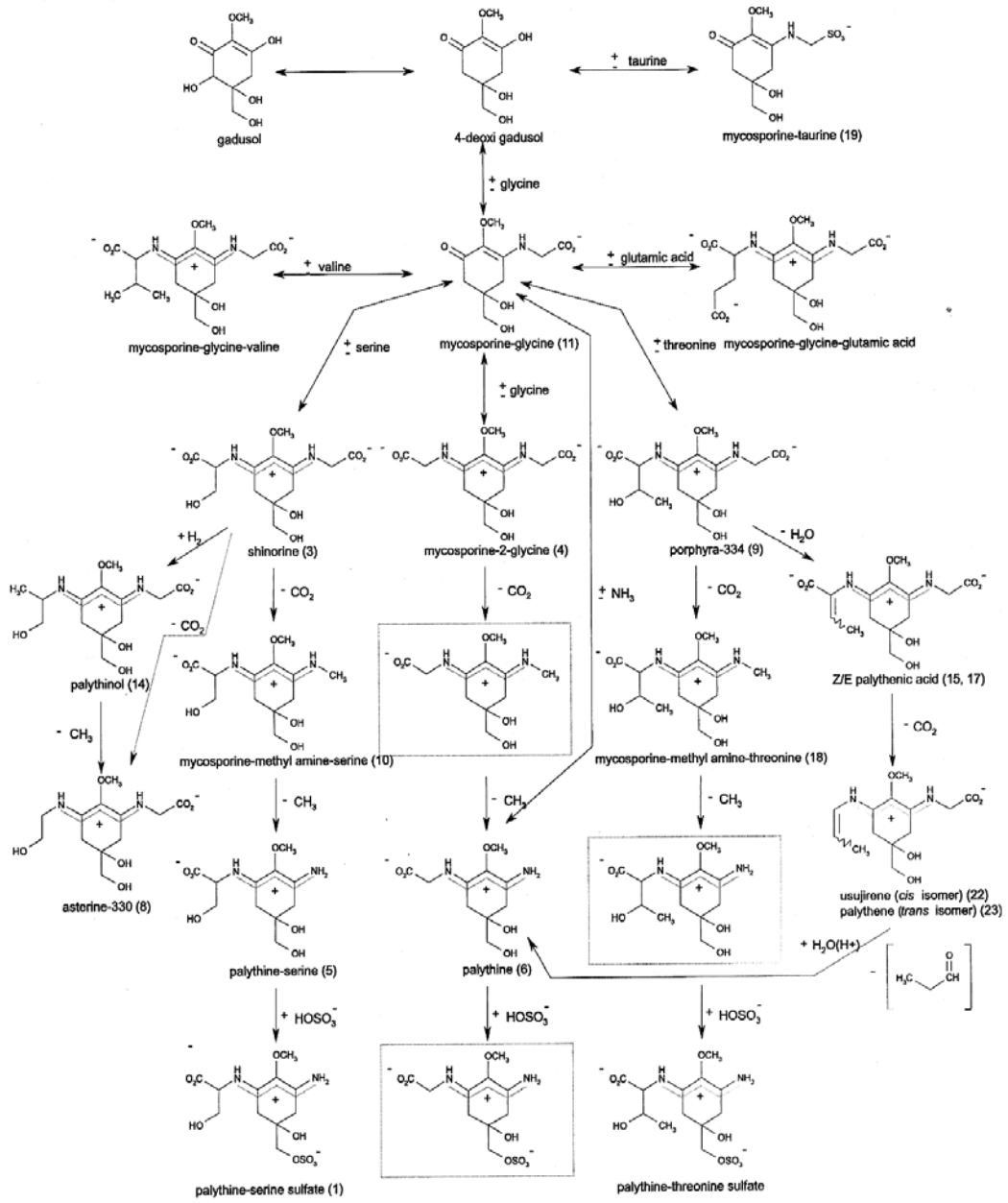


Figure 1. Some of the most common MAA's found in marine organisms. Modified from Carreto et al. (2005).

Table 1. Summary of Indo-Pacific reef corals mycosporine-like amino acids (MAA's) composition found in the literature. Abbreviations of MAA's: Mycosporine-Glycine (MG); Palythine (PI); Palythene (PE); Palythinol (PL); Asterina-330 (AS); Porhyra-334 (PR); Mycosporine-2Glycine (M2G); Shinorine (SH); Mycorporine-methylamine:threonine (MMT)

| Species | MG | PI | PE | PL | AS | PR | M2G | SH | MMT | Ref. |
|---|---|---|---|---|---|---|---|---|---|---|
| *Lobophyllia corymbosa* | + | + | | + | | + | + | + | | 1 |
| *L. hemprichii* | + | + | | + | | + | + | + | | 1,2 |
| *Favia pallida* | + | + | | + | | + | + | + | | 1 |
| *F. stelligera* | + | + | | + | | + | + | + | | 1 |
| *Porites rus* | + | + | | | + | | | + | | 1 |
| *P. lobata* | + | + | | + | | | | + | | 4 |
| *P. evermanni* | + | + | | + | | | | + | | 4 |
| *P. compressa* | + | + | | + | | | | + | | 4 |
| *Pavona cactus* | + | + | + | + | | + | | + | | 1 |
| *Fungia scutaria* | + | + | | + | | + | + | + | | 1,2 |
| *F. repanda* | + | + | | + | + | + | + | + | | 1,2,6 |
| *Montipora floweri* | + | + | | + | + | + | | + | | 1 |
| *M. hoffmeisteri* | + | + | | + | + | + | | + | | 1 |
| *M. hispida* | + | + | | + | + | + | | + | | 1 |
| *M. aequituberculata* | + | + | | + | + | + | | + | | 1 |
| *M. verrucosa* | + | + | | + | + | + | | + | | 1 |
| *M. faveolata* | + | + | | + | + | + | | + | | 1 |
| *M. caliculata* | + | + | | + | | + | | + | | 1 |
| *Acropora gemmifera* | + | + | | + | | + | + | + | | 1 |
| *A. formosa* | + | + | | + | | + | + | + | | 1,3 |
| *A. danai* | + | + | | + | + | + | + | + | | 1,2 |
| *A. paniculata* | + | + | | + | | + | + | + | | 1 |
| *A. microphthalma* | + | + | | + | + | | | | | 8 |
| *Pocillopora eydouxi* | + | + | | | | + | | + | + | 1,2 |
| *P. verrucosa* | + | + | | | | + | | + | + | 1 |
| *P. meandrina* | + | + | | | | + | | + | + | 1,2 |
| *P. damicornis* | + | + | | | | + | | + | + | 1,7 |
| *Stylophora pistillata* | | | | | | | | + | + | 5,7 |

1) Teai et al. (1997)
2) Teai et al. (1998)
3) Dunlap and Chalker (1986)
4) Shashar et al. (1997)
5) Banaszak et al. (2000)
6) Drollet et al. (1997)
7) Wu Won et al. (1995)
8) Shick et al. (1995)

Table 2. Summary of Caribbean reef corals mycosporine-like amino acids (MAA's) composition found in the literature. Abbreviations of MAA's: Mycosporine-Glycine (MG); Palythine (PI); Palythene (PE); Asterina-330 (AS); Porhyra-334 (PR); Shinorine (SH)

| Species | MG | PI | PE | AS | PR | SH | Ref. |
|---|---|---|---|---|---|---|---|
| *Madracis mirabilis* | + | | | | + | + | 1 |
| *Siderastrea radians* | + | | | | + | + | 1 |
| *Agaricia tenuifolia* | + | + | | | + | + | 1 |
| *A. agaricites* | + | + | | | | | 4 |
| *Diploria strigosa* | + | + | + | + | + | + | 1 |
| *Meandrina meandrites* | + | | | | | + | 2 |
| *Porites astreoides* | + | + | | + | | + | 3 |
| *P. porites* | + | + | | | + | + | 1 |
| *Montastraea annularis* | + | + | + | + | + | + | 1 |
| *M. faveolata* | + | + | + | + | + | + | 5,6,7,8 |
| *M. cavernosa* | + | + | + | + | + | + | 1,7 |

1) Banaszak et al. (1998)
2) Banaszak et al. (2000)
3) Gleason (1993)
4) Gleason and Wellington (1995)
5) Muszynski (1997)
6) Muszynski et al. (1998)
7) Lesser et al. (2000)
8) Corredor et al. (2000)

**Carotenoids**

Peridinin is the principal carotenoid of the dinoflagellates including the zooxanthellae (Dinophyceae, Division Pyrrophyta). There are also present P-457, β-carotene, diadinoxanthin, dinoxanthin, diadinochromes I and II, pyrrhoxanthin, astaxanthin, peridininol, diatoxanthin, and pyrrhoxanthinol (Bidigare, 1991). P-457 is the most polar carotenoid found in dinoflagellates; while dinoxanthin is a biosynthetic precursor of peridinin, and the diadinochromes are rearranged forms of diadinoxanthin (Johansen et al., 1974; Jeffrey et al., 1975, 1997, 1999).

Due to the presence in cells of photosensitizing molecules such as chlorophyll, flavins and aromatic amines, UV irradiation can cause the production in host animals and their endosymbiotic algae of reactive oxygen species, including superoxide ($O_2^{-\cdot}$), hydroxyl ($^{\cdot}OH$) radicals, and hydrogen peroxide ($H_2O_2$). Thus, some of the effects of UV can be indirect, mediated by these toxic forms of oxygen (Tyrrell, 1991). Pigments such as the β-carotene types absorb light, but do not transfer excitation energy to the reaction centers. Carotenoids may also act in stabilizing protein conformation in non-photosynthetic systems (Jeffrey et al., 1974). Light-harvesting by carotenoids in photosynthetic organisms involves the transfer of singlet excitation energy from carotenoids to chlorophylls in antenna pigment-protein complexes. Carotenoids also quench active oxygen species (Krinsky, 1971). Carotenoids may be involved in the dissipation of excess excitation energy from chlorophylls via singlet-singlet energy transfer to carotenoids, thereby preventing damage to the photosynthetic apparatus (Frank et al., 1996).

The xanthophyll cycle plays a role in the prevention of photoinhibitory damage to the photosynthetic apparatus. This cycle involves a light-driven reaction that transforms epoxy-containing xanthophylls into epoxy-free pigments (Hager, 1980). It has been found that the brighter the light to which the cells are exposed, the more the coral symbionts synthesize the carotenoids (Frank et al., 1996). In higher plants and chlorophytes, the xanthophyll cycle involves the pigments violaxanthin, antheraxanthin and zeaxanthin, where the formation of zeaxanthin helps in the process of non-radiative dissipation of excess light energy (Jeffrey et al., 1974). In phytoplankton and the Dinophyceae, the reactions involve the pigments diadinoxanthin and diatoxanthin (Liaaen-Jensen, 1978; Frank et al., 1996). Diadinoxanthin may also be present in both PSI and PSII light-harvesting complexes (Brown et al., 2000; Warner et al., 2002), and can make up to 20-30% of the total carotenoids (Johansen et al., 1974; Jeffrey et al., 1975). Diadinoxanthin also plays a role as light-harvester in some algae; hence, different pools of this pigment may be devoted to light harvesting and the xanthophyll cycle (Demers et al., 1991).

**GFP-like proteins**

Other compounds that have been ascribed to providing photoprotection to corals living under high-light conditions are the so-called Green Fluorescent Pigment-like proteins (GFP's) (Salih et al., 1998b, 2000). They also are believed to enhance photosynthesis under low-light conditions (Schlichter and Fricke, 1990), or both, depending on the position of the pigment relative to the zooxanthellae (Salih et al., 2000; Dove et al., 2001); but Mazel et al. (2003) found no relationship between GFP and photosynthesis. Mazel et al. (2003) found widely distributed GFP's in Caribbean coral

species, but did not found any significant correlation between depth and the GFP content of species such as *M. faveolata* and *M. cavernosa*. Although, as seen, GFP's might be another photoprotective mechanism against deleterious solar irradiances, the presence of these compounds was not analyzed in the present study.

**Variation of Zooxanthellae in Cnidarians**

The existence of zooxanthellae has been known since Brandt (1883) coined the term for the symbionts of radiolarians. In Cnidaria, the *Symbiodinium* cells are generally intracellular, located in cells of the endoderm and their elimination arises either from expulsion from the cell or from the cell death accompanied, under some circumstances by expulsion (Douglas, 2003).

Among zooxanthellate cnidarians, zooxanthellae density varies across species, habitat/depth, temporally, and with position within a colony. The symbiotic relationship operates very differently among coral species. Major characteristics of the symbiotic relationship include zooxanthellae density, size of zooxanthellae, rate of release of degraded zooxanthellae, and the relative rate of zooxanthellae division within the host (Stimson et al., 2002). The symbiosis of zooxanthellae with octocorals may be more conservative than that with scleractinians (Lasker, 2003); yet, since octocorals are not considered in this work, only the relationship between the zooxanthellae and the scleractinian corals is briefly discussed.

The phylogeny of *Symbiodinium* can be divided into several clades of subgenera, each of which contains as-yet-undetermined number of genetically distinct ecological "types" or species. The prevalence of particular symbionts across host taxa in both the

Pacific and the Caribbean provinces follows a classical Fisher log-normal distribution that is characterized by several very common types and many host-specific and/or rare types (LaJeunesse, 2002; LaJeunesse et al., 2003). The majority of the symbionts found in the Great Barrier Reef (>85%) belong to the phylogenetic clade C composed of closely related, yet ecologically and physiologically distinct types (Baker and Rowan, 1997; Savage et al., 2002a).

The comparison of symbiont and corals diversity shows an inverse relationship, perhaps as a consequence of more-rapid diversification of Caribbean vs. Indo-Pacific symbionts. Possibly because of environmental changes in the Caribbean after geographic isolation through the Quaternary period, a high proportion of Caribbean fauna became associated with symbiont taxa from two other distantly related clades (A and B) that rarely occurred in Pacific hosts (Baker and Rowan, 1997; LaJeunesse et al., 2003). "Caribbean symbioses" involving symbionts from clades A and B probably evolved during the environmental perturbations and extinctions that accompanied the Plio-Pleistocene transition (3.5-1.5 Mya). During this time, the Caribbean neotropics experienced a dramatic increase in faunal turnover and diversification. Many specialized Caribbean symbionts radiated after the coral extinctions of 1.6-0.8 Mya, whereas more of their counterparts from the GBR date further back, to the separation of the Atlantic and Pacific (LaJeunesse et al., 2003).

**Bleaching**

Bleaching is defined as the loss of color, arising from the partial to total elimination of the *Symbiodinium* population or degradation of algal pigments (Hoegh-

Guldberg, 1999). In this sense, bleaching is the extreme at one end of a continuum of variation in zooxanthellae density (Fitt et al., 2000). Cellular mechanisms which would result in reduced zooxanthellae densities in bleached corals include: degeneration of zooxanthellae *in situ*, release of zooxanthellae from mesenterial filaments, and release of algae within host cells detached from the endoderm in species with an advanced state of bleaching (Brown et al., 1995). "Normal" zooxanthellae can be defined as circular, with distinct cellular components such as nuclei, pyrenoids and assimilation bodies. In bleached corals, zooxanthellae appear "degraded" with a breakdown of cellular components and an overall loss of circularity.

While bleaching can occur naturally, it is an ecologically significant response resulting in reduced growth rates, suppression of sexual reproduction, impaired healing after mechanical damage, increased susceptibility to disease and, occasionally, mass mortality (Goenaga et al., 1989; Szmant and Gassman, 1990; Jones, 1997b; Wilkinson, 1999; Wilkinson et al., 1999). In fact, symbioses generally display seasonal variation in the density and pigment content of algal cells, with minimal values at the end of the season with the highest sea water temperatures. The longer the season of high sea water temperature, the more bleaching is predicted (Douglas, 2003). Coral bleaching can also occur during very high rainfall events as a result mainly of an increase in the water turbidity. Acevedo and Goenaga (1986) found an unusual bleaching in colonies of *Meandrina meandrites, Porites astreoides* and *Siderastrea siderea* at 20m depth in Peñuelas, Puerto Rico, after great quantities of rainfall during the storm season of 1985. While coral bleaching has been considered a disease (Rosenberg and Ben-Haim, 2002) or

not (Peters, 1984; Richardson, 1998), recently Weil (2004) presented evidence that bleaching can be referred to as a "non-pathogenic disease".

Coral bleaching and diseases, which have increased dramatically during the last few decades, may be a result of global warming (Wilkinson et al., 1999). In the 20$^{th}$ century, there was an average worldwide 1ºC rise in temperature, the largest in more than 1000 years, and a much larger rise has been predicted for this century (Bijlsma et al., 1995). The tolerance of the zooxanthellae to environmental factors such as temperature and irradiance varies among the different clades. For example, cells of clade C have a lower tolerance than clades A and B to elevated temperature/irradiance (Douglas, 2003). Nonetheless, Toller et al. (2001a) found differences in the type of *Symbiodinium* in the *M. annularis* complex with type E dominating high irradiance environments along with type C. Similarly, Toller et al. (2001b) experimentally reduced the number of zooxanthellae (by transplanting to shallow water) and allowing corals to recover. When depletion was not extreme, corals generally contained the same types of zooxanthellae as they did prior to treatment. After severe depletion, however, corals were always repopulated by zooxanthellae atypical for their habitat or for the coral species suggesting that zooxanthellae in *Montastraea* range from fugitive opportunists and stress-tolerant generalist (*Symbiodinium* A and E) to narrowly adapted specialists (*Symbiodinium* B and C), and may undergo succession and that coral-zooxanthella associations may or may not be re-established following disturbance, depending on the magnitude of zooxanthellar depletion (Toller et al., 2001b).

There are two predictions on the variation in susceptibility of *Symbiodinium* to bleaching (Douglas, 2003). First, clades A and D are anticipated to be favored over clade

C and possibly B in some animal species subjected to bleaching episodes. This may result in a shift in the dominant clade to A/D in some species, and increased abundance of species capable of forming a symbiosis with these clades, since many symbioses may be described more aptly as "selective" than as "specific" in their choice of algal partner. The abundance of a symbiosis is also predicted to be influenced by the impact of the acquired *Symbiodinium* cells on the performance of the host, including the long-term effects on growth, reproduction and resistance to disease. Second, symbioses that bleach in response to elevated temperature/irradiance and survive are anticipated to be more tolerant of subsequent episodes of elevated temperature/irradiance without a shift in the complement of *Symbiodinium* cells, as a result of physiological acclimation. The latter has been known as the Adaptive Bleaching Hypothesis (Buddemeier and Fautin, 1993; Buddemeier and Smith, 1999; Douglas, 2003).

Warner et al. (1999) found that dinoflagellates of bleaching-resistant corals have significantly greater capacity for maintenance of PSII at elevated temperatures than those of thermal-sensitive corals. Recently, Baker et al. (2004) hypothesized that corals containing unusual algal symbionts that are thermally tolerant are much more abundant in reefs that have been severely affected by recent climate change. This adaptive shift indicates that these devastated reefs could be more resistant to future thermal stress, resulting in significantly longer extinction times for surviving corals that had been previously assumed; and that the symbiont changes are a common feature of severe bleaching events, and these adaptive shifts will increase the resistance of these recovering reefs to future bleaching (Buddemeier and Fautin, 1993; Baker et al., 2004).

# EXPERIMENTAL SETUP AND CHARACTERIZATION OF THE UNDERWATER LIGHT FIELD

This work was divided into two separate experiments in order to examine the metabolic responses of the studied species under two completely different light regimes. This section presents a general description of the experimental setup for the two different experiments performed with the two species. Additionally, this section presents a general description of the underwater light field at the shallow back-reef habitat of San Cristóbal Reef and at the Old Buoy site at La Parguera shelf edge. The underwater light field is also described for an outdoor open-system aquariums setup that was used during the second experiment. A specific description of each procedure used either in the field or in the laboratory for biological sampling and analysis can be found at each particular chapter.

## Experimental setup

### General studied species description

*Acropora cervicornis* and *Porites furcata* (Figure 2) have similar geographic and local distributions. Both species are circumscribed to the tropical Atlantic waters, including the Caribbean Sea, the Bahamas, and the Gulf of Mexico. Locally, both species thrive in shallow clear reef waters between 0-5m, but it is not uncommon to find colonies at depths up to 20m. Both species are perforate ramose corals (Barnes, 1982) with relatively rapid growth rates (Gladfelter, 1982; Edmunds and Davies, 1989). For example, in *A. cervicornis*, the extending axial corallite attains its maximum diameter in less than a day (Gladfelter, 1982). There is an apparent diel pattern in the deposition of

the skeleton of this species (Chalker, 1977; Gladfelter, 1983). Both species usually show polyp expansion during daytime. In general, highly phototrophic genera with medium or small polyps, such as those of *Acropora* and *Porites*, respectively (Edmunds and Davies, 1989; Davies, 1991), seem to be more successful in competing for light by outgrowing competitors, while the more heterotrophic genera with larger polyps, such as *Montastraea*, can persist in the understory layer (Falkowski et al., 1990). *A. cervicornis* and *P. furcata* differ in their respective reproductive patterns (*A. cervicornis* is a hermaphroditic broadcast spawner and *P. furcata* is a gonochoric brooder); nonetheless, both species have their peak reproductive season during the summer (Soong, 1991).

Figure 2. Photos of studied species: *Acropora cervicornis* (upper photo) and *Porites furcata* (lower photo).

**Study sites description**

A. San Cristóbal Reef (17°56'41"N; 067 °04'38"W) is located in La Parguera Reef Platform, approximately 2 miles off the Magueyes Island Field Station (University of Puerto Rico, Mayagüez Campus, Department of Marine Sciences) (Figure 3). Here, *Acropora cervicornis* and *Porites furcata* dominate the coral cover at the shallow waters of the back-reef zone in less than 5m. Other coral species present in lower abundances are: *Acropora prolifera, Montastraea annularis, Porites astreoides, Favia fragum, Diploria clivosa, D. strigosa* and *D. labyrinthyformis*. Other benthic components include calcareous algae and octocorals. Relatively calm and clear water is found year long in this area.

B. The Old Buoy site is located at La Parguera shelf edge (17°53'11"N; 066°59'51"W) approximately 6 miles off Magueyes Island (Figure 3). The site is characterized by a spur and groove topography with medium-sized colonies of *M. faveolata, M. franksi, M. cavernosa, Colpophyllia natans, Porites astreoides, Agaricia* spp. and *Mycetophyllia* spp. dominating the scleractinian fauna. Colonies of *A. cervicornis* can be found scattered in the area, but the coverage is less than that of the above-mentioned species. Depth in the shelf varies between 19m on the platform and 24m at the shelf edge.

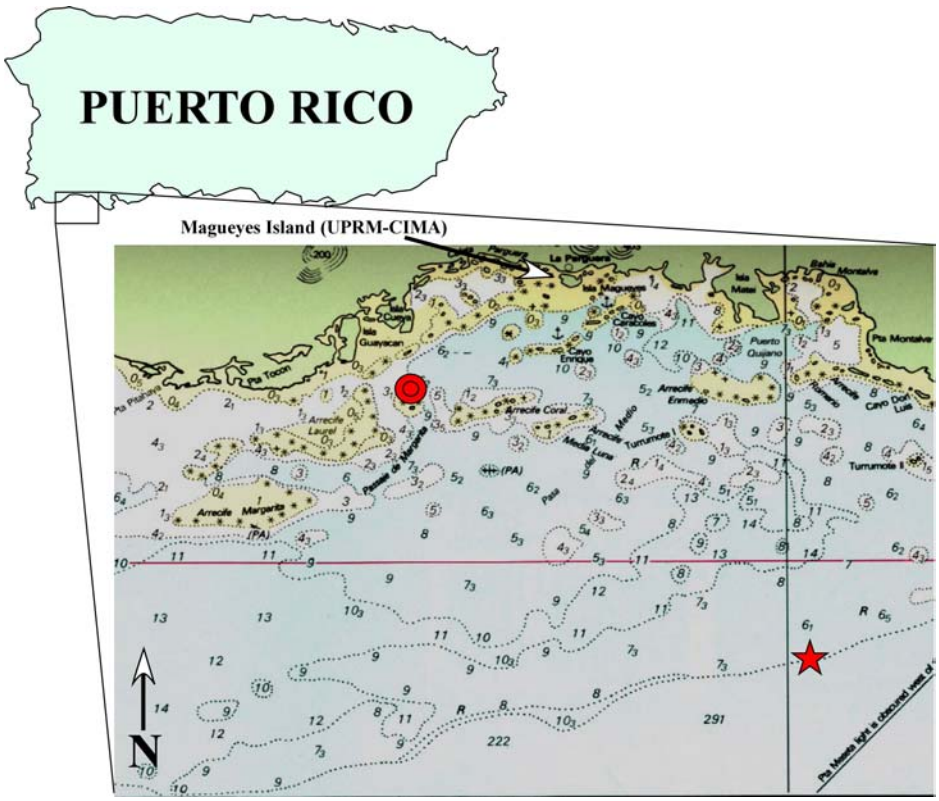


Figure 3. Study sites location. The circles indicate the position of the back-reef area of San Cristóbal Reef and the star indicates the Old Buoy shelf edge area in La Parguera Reef Platform.

**UV-Exclusion Experiment**

The first experiment, conducted during April 6 – July 3, 2001, consisted of an UV-Exclusion Experiment (named UV-E hereafter). In this experiment, 18 colonies of *Acropora cervicornis* and 18 colonies of *Porites furcata* were subjected to depleted UVR and reduced PAR levels received at actual colony living depths (1.5m) at San Cristóbal Reef in La Parguera, Puerto Rico. Additionally, six colonies of each species were placed in a $4m^2$ quadrat spaced 5m away from the location of the treatments and were used as controls. The controls were exposed to normal daily UVR and PAR doses received at 1.5m depth. All colonies of both species were collected on-site at a 5m distance to their

nearest intraspecific neighbor to avoid pseudoreplication due to clone mates. All colonies (controls and treated) were tied to 1.25cm diameter PVC tubes located in cement platforms to avoid contact with the sandy bottom of the area. All other physical factors remained unaltered (salinity, water motion, temperature, etc.).

To block most of the UVR and some of the PAR received at 1.5m at San Cristóbal Reef, three hemispherical open-ended steel rod frames (1m width X 1m length X 1m height) were constructed for each treatment. On one treatment, a Hyzod® acrylic panel covered three of the steel frames. This material excludes 99% of the UVR and 23% of PAR. On the second treatment, a Saran® mesh (absorbs 95% UVR and 82% PAR) was tied to the remaining three steel frames. Six colonies of each species were located under each hemispherical frame. All frames were located at the same depth as the control colonies (1.5m). Figure 4 shows a diagram of the experimental setup for the UV-E Experiment.

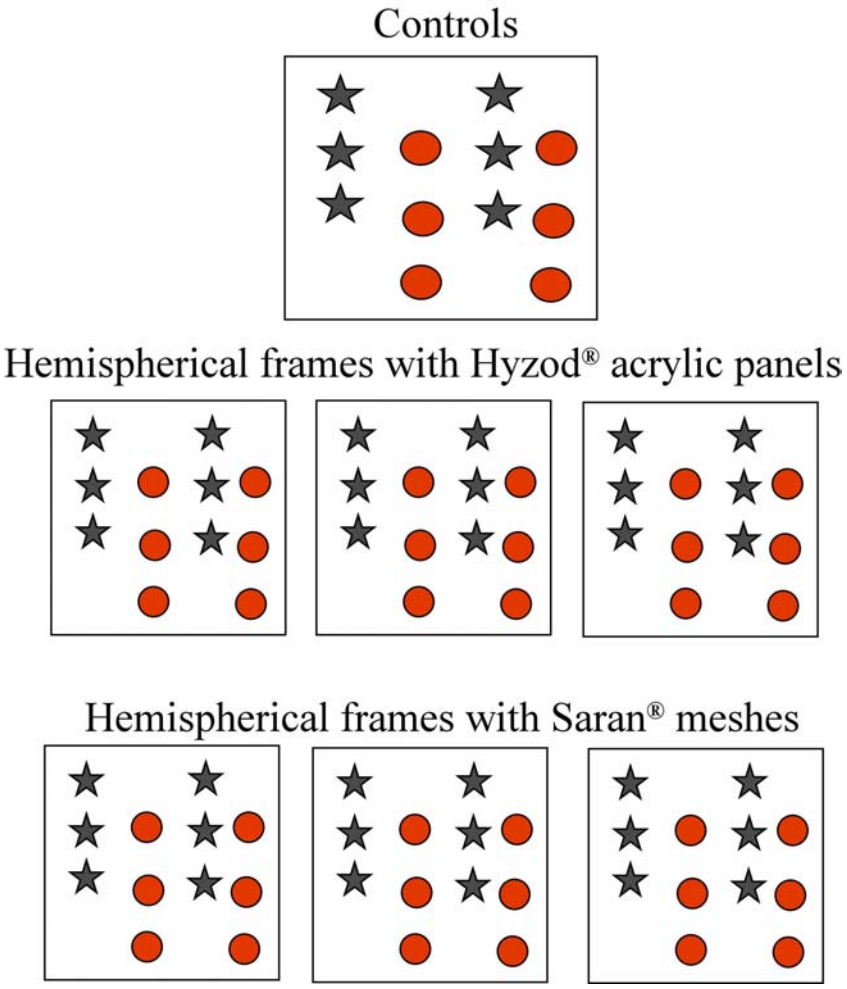


Figure 4. Schematic representation of the experimental setup for the UV-E Experiment. Circles represent colonies of *A. cervicornis* and stars represent colonies of *P. furcata*.

**UV-Supplementation Experiment**

The UV-Supplementation Experiments (named UV-S hereafter) were conducted during March 20 – August 15, 2003. Two different UV-S experiments were carried out depending on the species used. The first one consisted of exposing colonies of *Acropora cervicornis* to an increase in UVR and PAR in the field by transplanting ten colonies living at La Parguera shelf edge (20m depth) and ten colonies from El Palo Reef (8m depth) to the back-reef area of San Cristóbal Reef at 1.5m depth. Ten colonies of *A. cervicornis* living at San Cristóbal Reef at 1.5m and ten colonies from El Palo Reef (8m

depth) were also transplanted to the shelf edge at 20m. Additionally, ten colonies were used as controls at San Cristóbal (1.5m depth) and ten colonies were used as controls at the shelf edge (20m depth). All studied colonies (controls and transplants) were tied to plastic-covered cyclone fence wires to avoid contact with the bottom. These cyclone fence wires were held in place with cement blocks. Figure 5 shows a diagram of the experimental setup used with the *A. cervicornis* transplants.

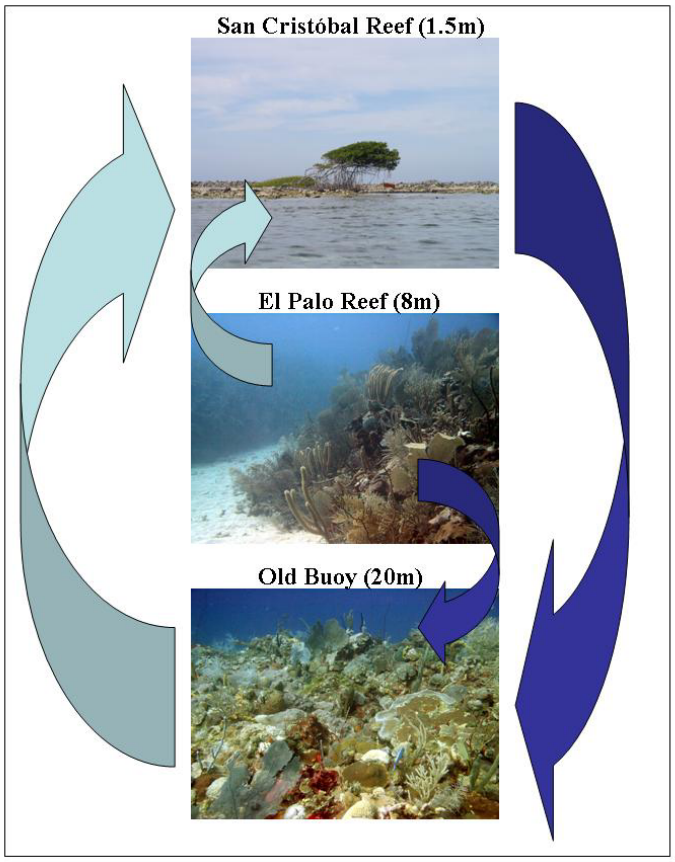


Figure 5. Schematic representation of the *A. cervicornis* transplants. Dark blue arrows represent transplants from shallow to deeper waters and clear blue arrows represent transplants from deep to shallower waters. Each arrow regarding of size represent the transplantation of ten colonies. Ten additional colonies collected on-site were used as controls for San Cristóbal Reef and the Old Buoy.

The second UV-S experiment consisted in supplementing UVR with an artificial light system over an open-system running seawater outdoor aquariums setup built at the Magueyes Island Field Station. Nine 75-liter aquariums were set in three different water tables (three aquariums per table). All the colonies of *Porites furcata* were collected at the back-reef habitat of San Cristóbal Reef at 1m depth. Three colonies of *P. furcata* were transplanted to each aquarium. The colonies transplanted to the aquariums located in a specific water table were exposed to the same treatment. Hence, each aquarium located in a table served as a replicate of the one next to it (n=3/treatment). Nine colonies were exposed to mimicked levels of UVR and PAR received at collection depth by placing a neutral density mesh over the aquariums and served as controls. Nine colonies were exposed to enhanced UV radiation by supplementing the solar incident levels of UVR with Q-Panel 313nm UV fluorescent lamps located 0.5m over the top of the aquariums. These lamps were controlled by and electric timer and turned on for one-hour during the maximum sun zenith angle hours (11:30-12:30). To contrast with the results obtained by either enhancing the amount of UVR reaching the colonies or the control colonies, the remaining nine colonies were exposed to depleted UVR in three aquariums located under a Hyzod® acrylic panel similar to the ones used during the UV-E Experiment. In this sense, both scenarios (enhanced UVR and depleted UVR) could be examined and compared to the controls at the same time. Figure 6 shows a diagram of the outdoor aquariums setup at Magueyes Island.

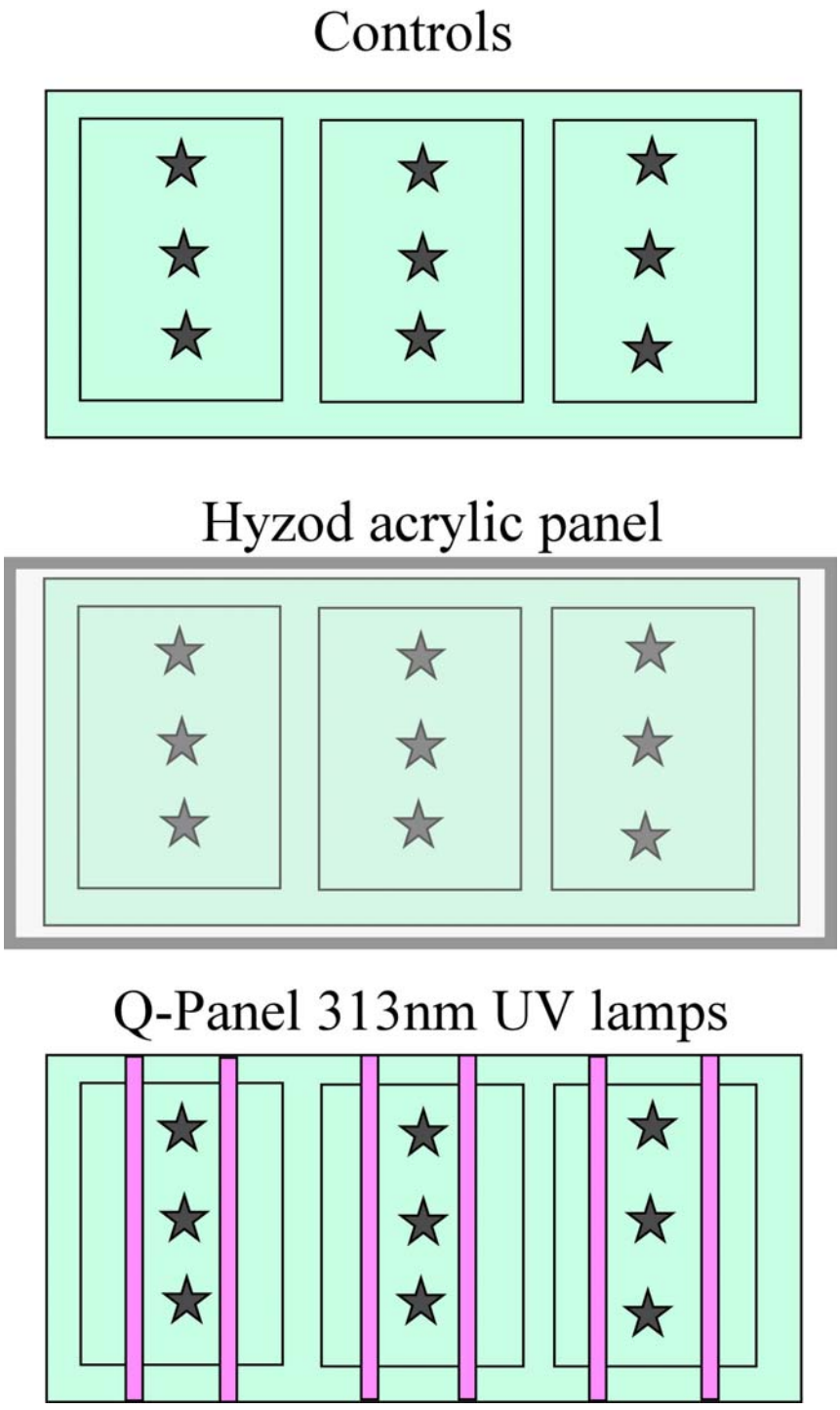


Figure 6. Schematic representation of the experimental setup used with the outdoor aquariums. Stars represent single *P. furcata* colonies. Vertical light violet bars represent Q-Panel 313nm UV lamps.

**Statistical analyses used for biological data**

All the biological data collected during both, UV-E and UV-S Experiments, regarding linear extension rates, skeletal density, fecundity, MAA's, and photosynthetic pigments concentrations were tested for normality and equality of variances using a Bartlett's Test with a Bonferroni 95% confidence intervals. Data was log-transformed whenever unequal variances were found to comply with the Fully Nested Analysis of Variance (ANOVA) assumptions. For all the biological data, a Fully Nested ANOVA was performed to test for statistical differences among treatments. A posteriori Tukey Test with Pairwise Comparisons was used to distinguish which treatments were different from others (Ott and Longnecker, 2001). Statistical significance was set at all tests at $\alpha = 0.05$.

**Ultraviolet Radiation (UVR) and Photosynthetically Active Radiation (PAR) Measurements**

Surface irradiance measures were obtained with a ground-based radiometer (Biospherical Instruments GUV-511) located at Magueyes Island. This radiometer contains four discrete bands at 305, 320, 340 and 380nm, and an additional channel that integrates downwelling irradiance in the visible region (400-700nm: PAR). The GUV-511 is configured to record downwelling irradiance at 5 minute intervals.

The experimental time period for both experiments coincided with the highest UVR reaching La Parguera reef platform surface waters as measured by the Magueyes Island UV monitoring station (Figure 7). Data from September to December of 2001 were not available due to instrumental problems.

Average downwelling irradiance in the UV region shows a typical bell-shaped curve with higher doses of both UV-B and UV-A occurring at noon (Figure 8). In general, the data shows an increase in monthly UVR doses from March to August and a marked decline after September. The decrease observed during April, 2003 is an artifact of few data points since only the first week was recorded during this month due to instrumental problems. The variations observed between 8:00-10:00 were due to the presence of scattered clouds during those hours.

Downwelling irradiance (Ed) of both UVR and PAR was measured at San Cristóbal Reef with an Optronics OL-754 spectroradiometer on June 20, 2001. Due to logistical problems involved with the movement of the Optronics OL-754 spectroradiometer, it was decided to make just one measurement of Ed near the middle of the experiment. Measurements of Ed were taken at water surface and at 1.5m depth (controls) with a $2\pi$ cosine collector located inside and underwater integrating sphere. Measurements of Ed were also taken under the two experimental treatments (Hyzod® and Saran®). UVR and PAR irradiances and doses reaching the surface per unit area were calculated following the procedures of Detrés et al. (2001). Briefly, daily surface or above-water UVR and PAR were calculated by integrating the daily irradiance measured with each band of the GUV-511 (305, 320, 340, 380nm, and PAR). The incident irradiance received at the reference colonies in the study site (1.5m) was then calculated using:

$$Ed_z = Ed(0^-)e^{-Kdz} \qquad (1)$$

where, $Ed(0^-)$ is the downwelling irradiance just below the surface, Kd is the vertical attenuation coefficient, and z is depth. Ed ($0^-$) was obtained with:

$$Ed(0^-, \lambda) = 1.03[0.97Ed(0^+, \lambda)] \quad (2)$$

where, 1.03 is the refraction index of seawater, 0.97 is the average transmittance across the air-sea interface, and $Ed(0^+, \lambda)$ is the surface irradiance obtained by the GUV-511 radiometer on land (Figure 1.5). Since the Magueyes Island UV Monitoring Station is near sea level, these values were used for above-surface Ed [$Ed(0^+)$] in the La Parguera reef platform.

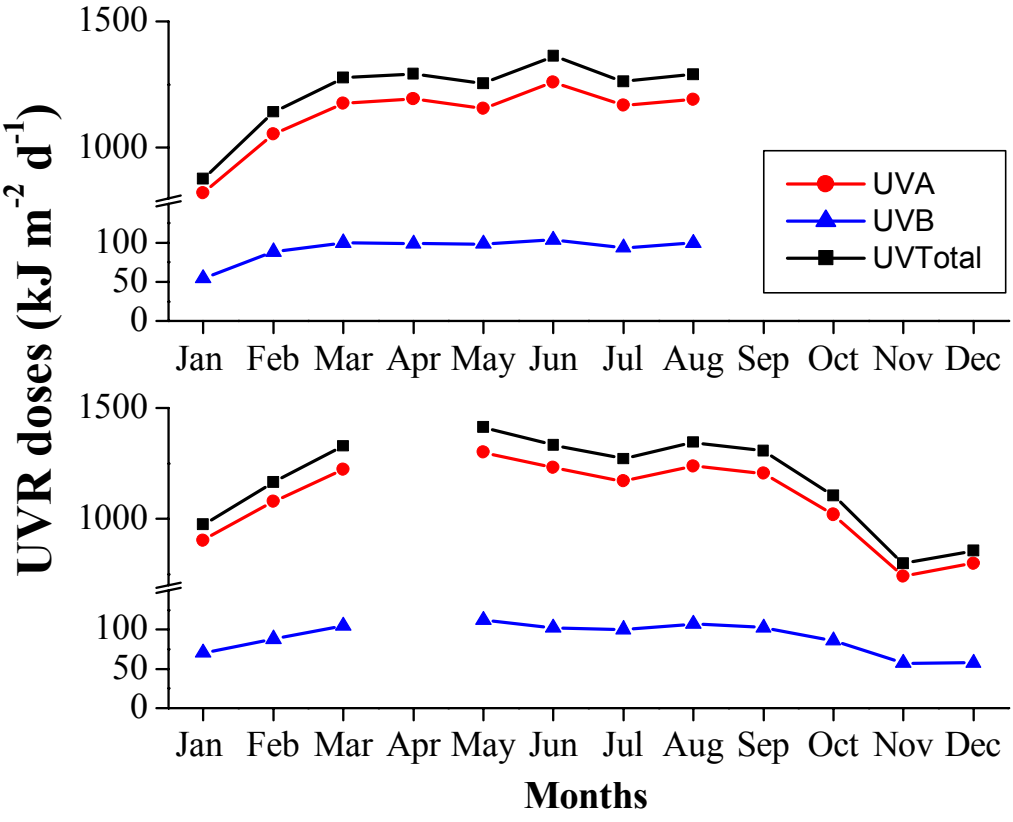


Figure 7. Average UVR doses received at the ocean surface calculated from the data collected by the GUV-511 radiometer at Magueyes Island, La Parguera, Puerto Rico: A) 2001, B) 2003. Data for April 2003 could not be obtained due to instrumental problems.

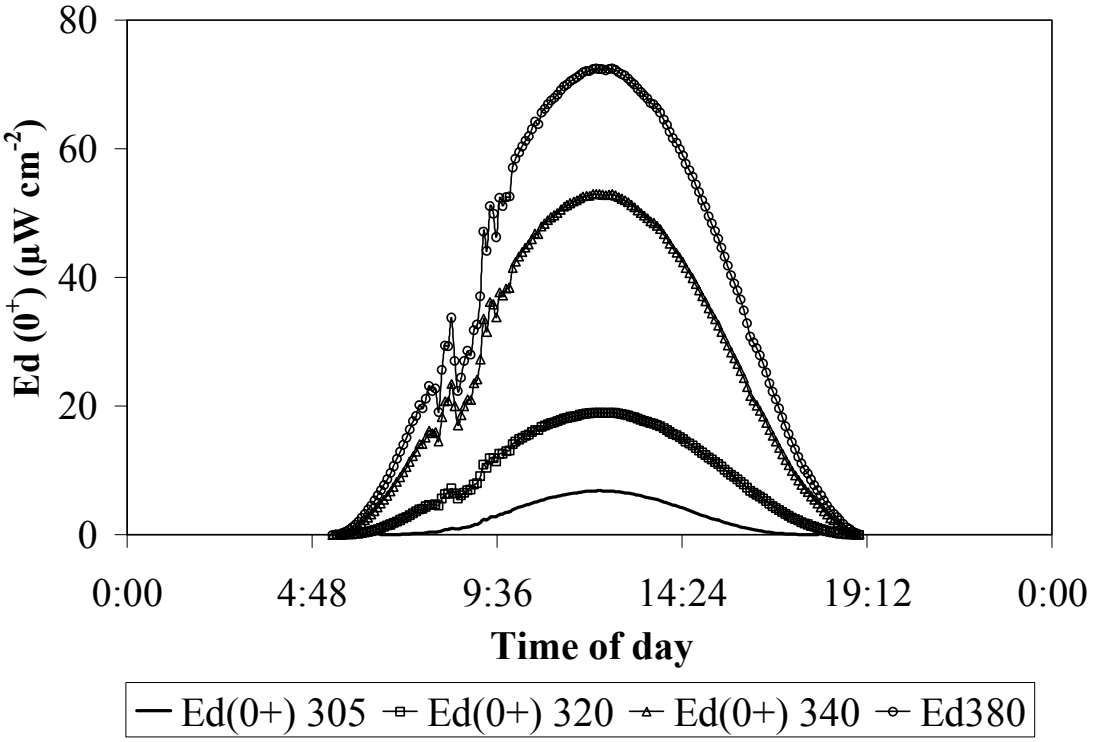


Figure 8. Average downwelling irradiance just above the surface [$Ed(0^+)$] measured by the four UVR bands of the GUV-511 radiometer at Magueyes Island the same day (June 20, 2001) spectral field measurements were taken with the Optronics OL-754 spectroradiometer. Noise between 8:00-10:00 was caused by presence of scattered clouds.

The diffuse attenuation coefficient (Kd) for UV-A, UV-B, and PAR was calculated during the UV-E Experiment from the measurements taken at the site with the Optronics OL-754 spectroradiometer just below the surface and at sampling depth (1.5m) and under the two experimental treatments (Hyzod® and Saran®) using:

$$Kd = 1/(Z_2-Z_1)[\ln(Ed_2/Ed_1)] \quad (3)$$

where, $Ed_1$ and $Ed_2$ are the downwelling irradiances measured at depths $Z_1$ and $Z_2$, respectively.

Daily doses of UV-A and UV-B were obtained by integrating daily irradiance values measured at 305, 320, 340, and 380nm for the length of the experiment. The data from the 305 and 320nm bands were used to estimate UV-B and the data from the 340 and 380nm bands were used to estimate UV-A, following the equations of Orce and Helbling (1996):

$$UV\text{-}B = 59.5(Ed_{305}) + 4.1(Ed_{320}) \quad (4)$$

and,

$$UV\text{-}A = 87.4(Ed_{340}) - 2.4(Ed_{380}) \quad (5)$$

where, $Ed_{305}$, $Ed_{320}$, $Ed_{340}$ and $Ed_{380}$ correspond to the downwelling irradiance measured by the 305, 320, 340, and 380 bands, respectively.

Changes in attenuation among treatments are reflected in the daily UVR doses received by the experimental colonies (Table 3). UVR and PAR attenuation showed to be wavelength-dependant (Figure 9) either at control depth or under the two treatments. Light attenuation in the PAR region was higher under the Saran® mesh, while only slight differences can be seen among treatments in the UVR region (Figure 10).

Table 3. Estimated daily UV radiation doses (kJ $m^{-2}$ $day^{-1}$) received at sampling depth (Control) and under the two experimental treatments during the UV-E Experiment. UVT = total UVR.

| Treatment | UV-B (kJ $m^{-2}$ $day^{-1}$) | UV-A (kJ $m^{-2}$ $day^{-1}$) | UVT (kJ $m^{-2}$ $day^{-1}$) |
|---|---|---|---|
| Control | 13.6 | 360.1 | 373.7 |
| Hyzod | 0.1 | 6.4 | 6.5 |
| Saran | 0.4 | 20.6 | 21.0 |

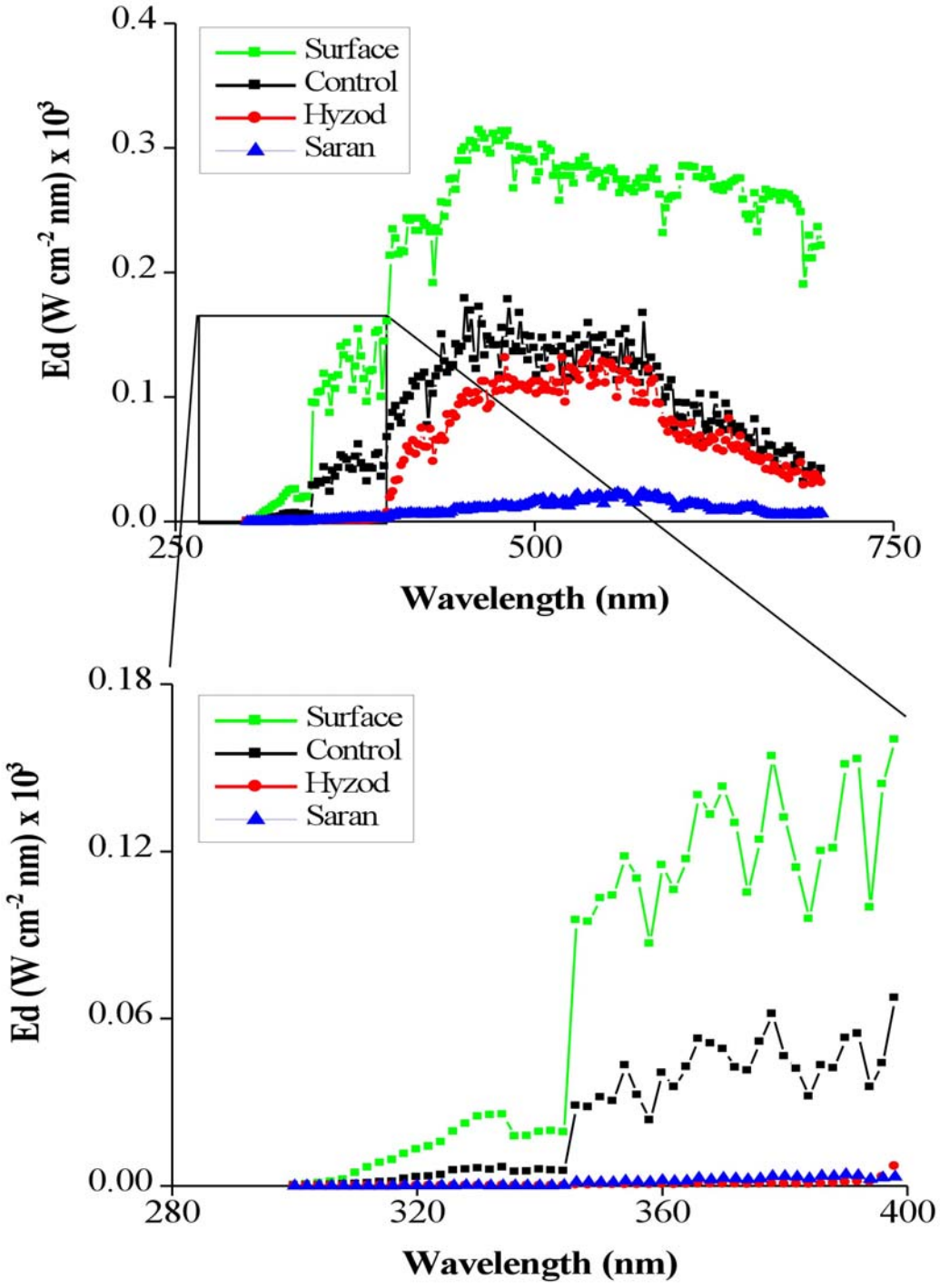


Figure 9. Downwelling irradiance (Ed) measured with the Optronics OL-754 spectroradiometer at 1.5m depth and at the surface at San Cristóbal Reef (June 20, 2001).

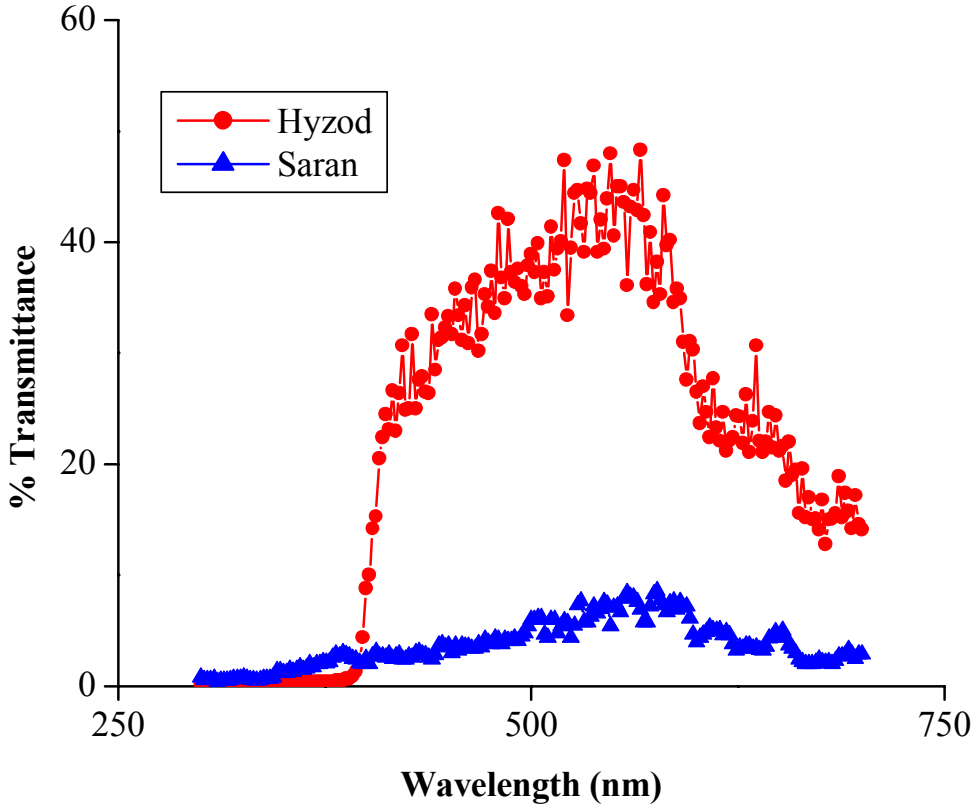


Figure 10. Transmittance of materials used to partially block UVR and PAR reaching treated colonies of both species. Transmittance was calculated using the measurements obtained at the sampling depth (1.5m) at San Cristóbal Reef.

The penetration of UVR and PAR was also measured at the Old Buoy site and San Cristóbal Reef at the beginning, middle, and end of the UV-S experiment. Total UVR levels at the sampling sites were obtained with a Solar Light radiometer connected to an underwater 2π cosine collector. Measurements were recorded at 1m intervals between surface and 10m depth and every 2m between 10-20m depth at the Old Buoy site and at 0.5m intervals at San Cristóbal Reef. PAR measurements were obtained at the same depths intervals with a LiCor 182s cosine collector connected to a LiCor 1400 data

logger. Both measurements (UVR and PAR) were later used to obtain Kd values for both sites as during the UV-E Experiment.

The unweighted downwelling irradiance (i.e. underwater downwelling irradiance without normalization by above-water Ed measurements) for the total UVR region ($Ed_{UVR}$) at the shelf edge (Figure 11) and at San Cristóbal Reef (Figure 12) shows an exponential decay similar to that of $Ed_{PAR}$. An additional $2\pi$ cosine collector located on top of the boat ceiling to correct for atmospheric changes during the sampling of $Ed_{PAR}$. Therefore, $Ed_{PAR}$ underwater measurements were normalized by simultaneous above-water Ed measurements in order to account for possible atmospheric light field changes during the measurements. While UVR decay is higher during the first 4m of water, both UVR and PAR show a similar decrease with depth below 8m, possibly due to higher concentrations of particulate (i.e. sediments, phytoplankton) and dissolved organic matter (DOM) and by the influence of wave action in the first layers of the water column.

The vertical attenuation coefficient for total UVR ($Kd_{UVR}$) were approximately two-three times higher than those of PAR ($Kd_{PAR}$) at the San Cristóbal back–reef area (Figures 11 and 12; Table 4). However, both $Kd_{UVR}$ and $Kd_{PAR}$ decreased at the shelf edge showing an increase in water transparency compared to the nearshore reef. Nevertheless, both Kd coefficients show some variations at different times of year (Table 4). The results compare to those of Dieppa-Ayala (1996) who found similar Kd measures in oligotrophic waters off La Parguera between March-June and no significant variations in $Kd_{PAR}$.

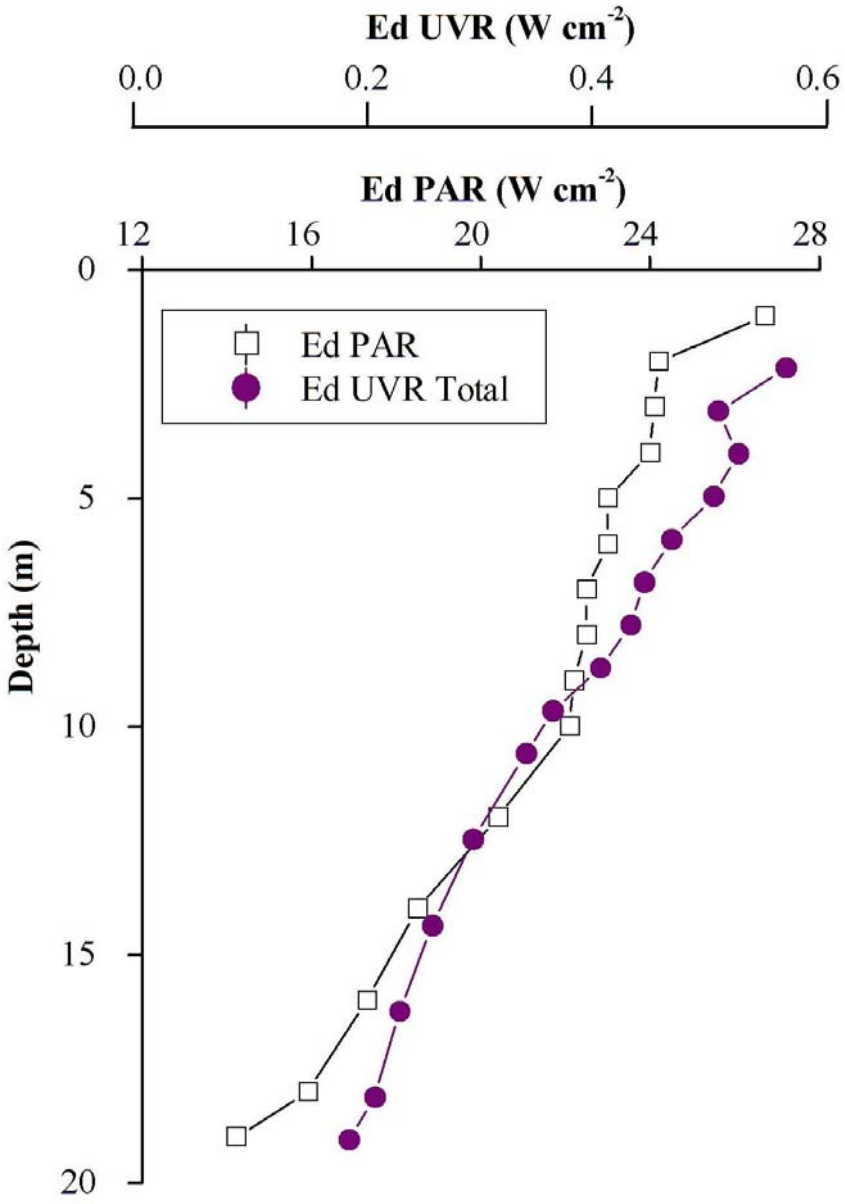


Figure 11. Downwelling irradiance (PAR and UVR) measured at the Old Buoy site during the UV-S Experiment. $Ed_{PAR}$ was corrected to atmospheric conditions. $Ed_{UVR}$ values are unweighted.

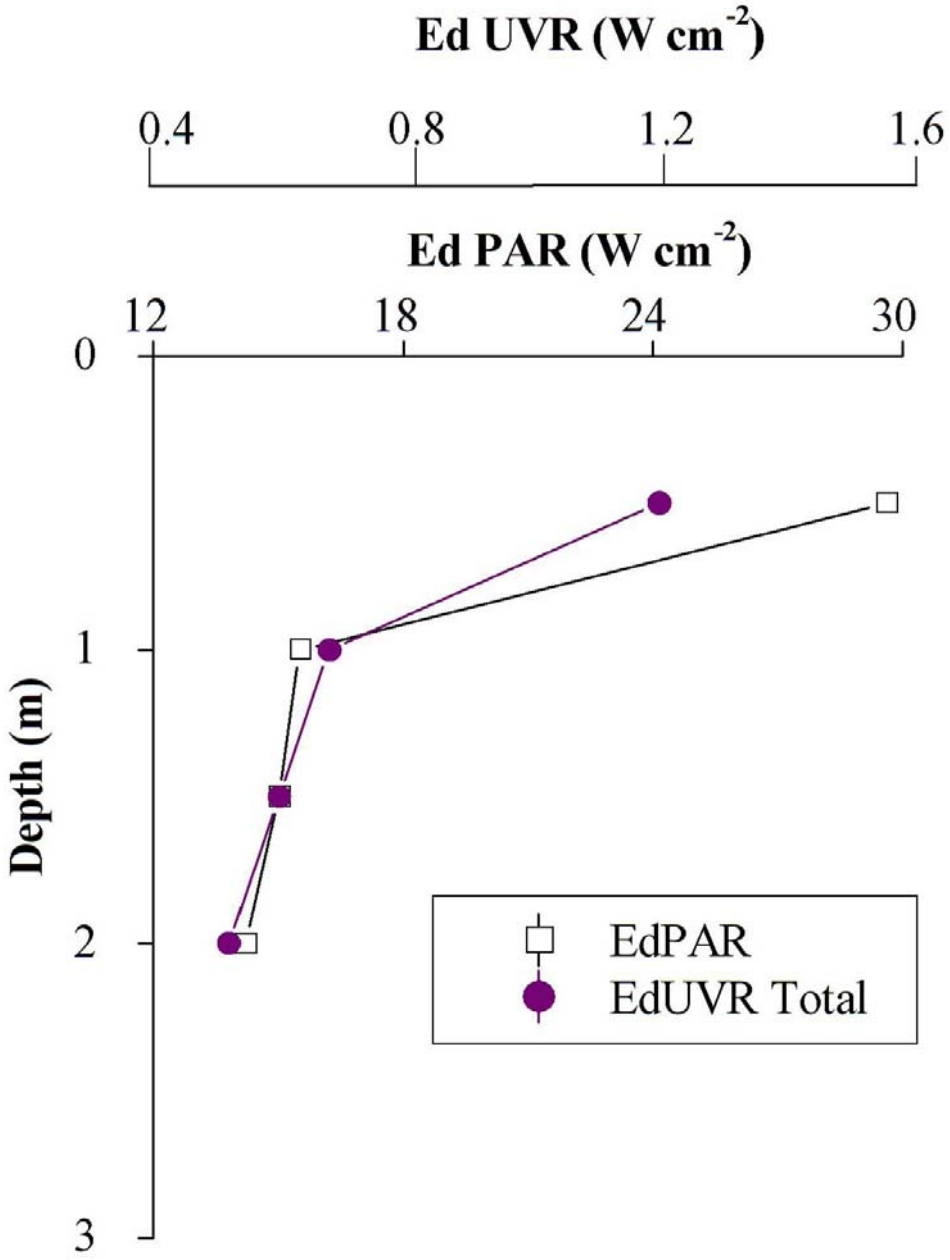


Figure 12. Downwelling irradiance (PAR and UVR) measured at San Cristóbal Reef during the UV-S Experiment. $Ed_{PAR}$ was corrected to atmospheric conditions. $Ed_{UVR}$ values are unweighted.

Table 4. Vertical attenuation coefficients (Kd) measured for total UVR and PAR at study sites at the beginning and end of the UV-S-Experiment.

| **Study Site** | **Kd (UVR) ($m^{-1}$)** | | **Kd (PAR) ($m^{-1}$)** | |
|---|---|---|---|---|
| | **20-Mar-03** | **31-July-03** | **20-Mar-03** | **31-July-03** |
| San Cristóbal Reef (1m) | 0.27 | 0.19 | 0.09 | 0.09 |
| Old Buoy (20m) | 0.13 | 0.16 | 0.06 | 0.08 |

An Optronics OL-754 spectroradiometer equipped with an underwater integrating sphere and a $2\pi$ cosine collector was used to obtain spectral measurements of UVR and PAR in the outdoor aquarium setup. Measurements were obtained in the controls, the Hyzod® and UV treatments (enhanced with Q-Panel 313nm lamps) during a clear-sky day just before and after the UV-S Experiment. The neutral density mesh located above the control aquariums reduced the levels of UVR and PAR received and compensated for the reduced height in water column present over the control colonies in the outdoor aquariums setup (Figure 13). UVR doses at each treatment were obtained as in the UV-E Experiment using the equations 4 and 5 with the measurements obtained with the GUV-511 radiometer. Values for total UVR doses were obtained as in the UV-E Experiment.

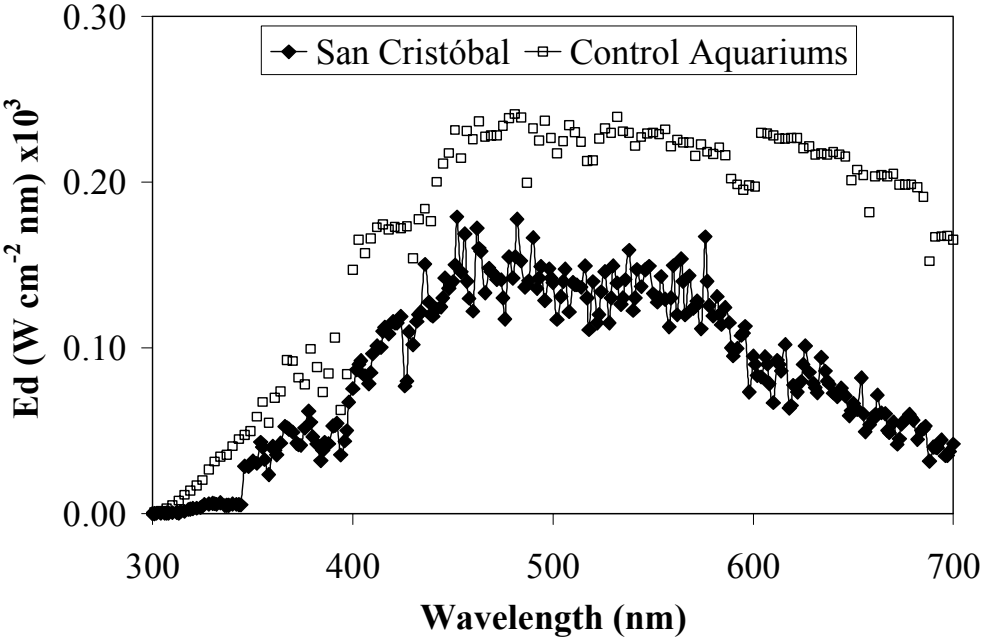


Figure 13. Comparison of incident downwelling irradiance at the control aquariums vs. at the back-reef area of San Cristóbal Reef (1.5m depth).

Total UVR was increased by 9.3-22.2% in the aquariums held under the artificially-enhanced UVR. This variation reflects the lamps decay due to usage. Individually, UV-B was enhanced between 4.8-13.7%, and UV-A between 4.5-8.5%. This is reflected in the respective Ed curves (Figures 14 and 15). There was no net change in the levels of UVR in the control aquariums (-0.20% change between July and March, 2003). Hence, by the end of the experiment the difference in UVR doses between the controls and the aquariums submitted to enhanced UVR was reduced.

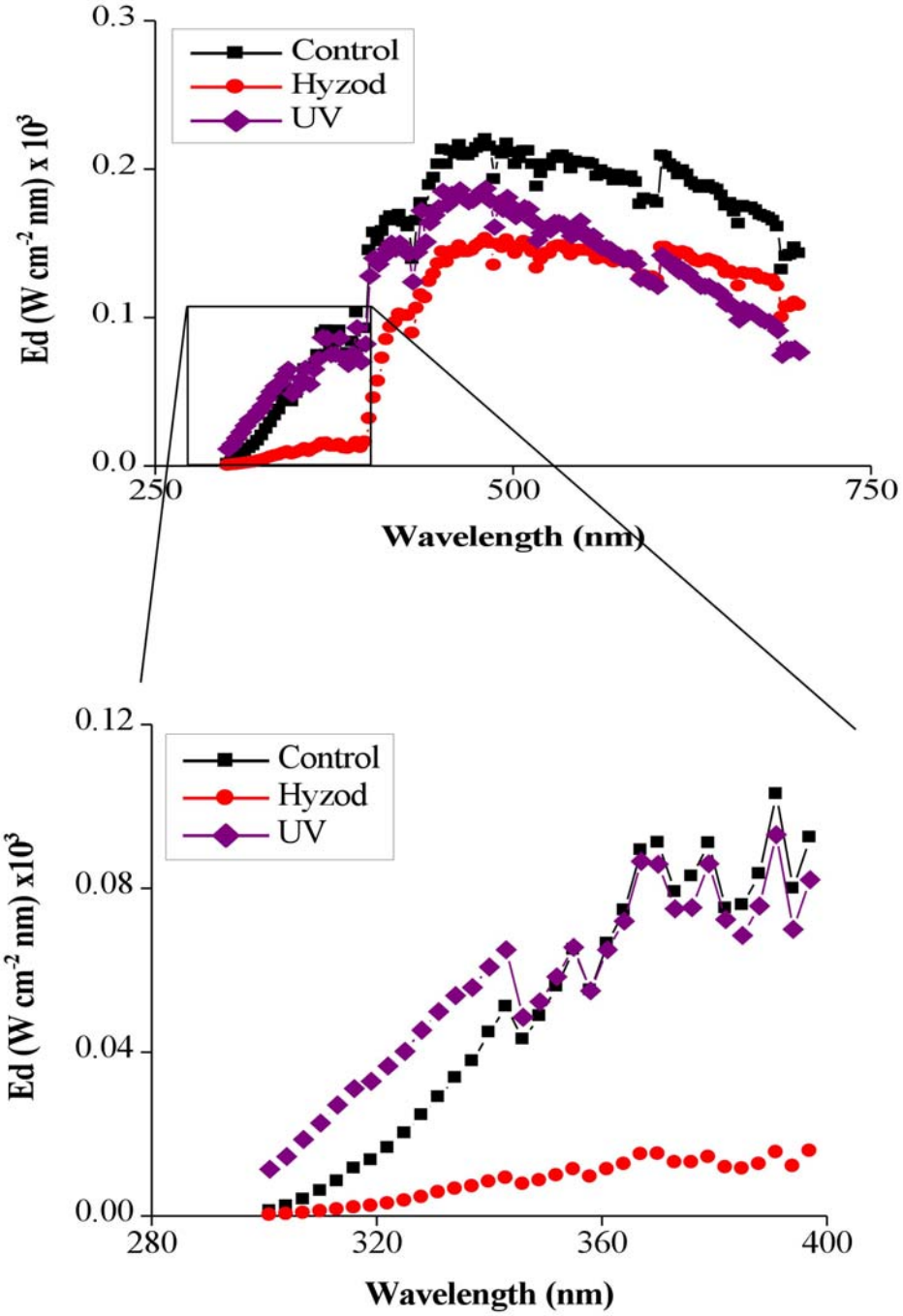


Figure 14. Downwelling irradiance (Ed) measured with the Optronics OL-754 spectroradiometer in the outdoor aquariums at Magueyes Island just before the beginning of the UV-S Experiment (March 16, 2003).

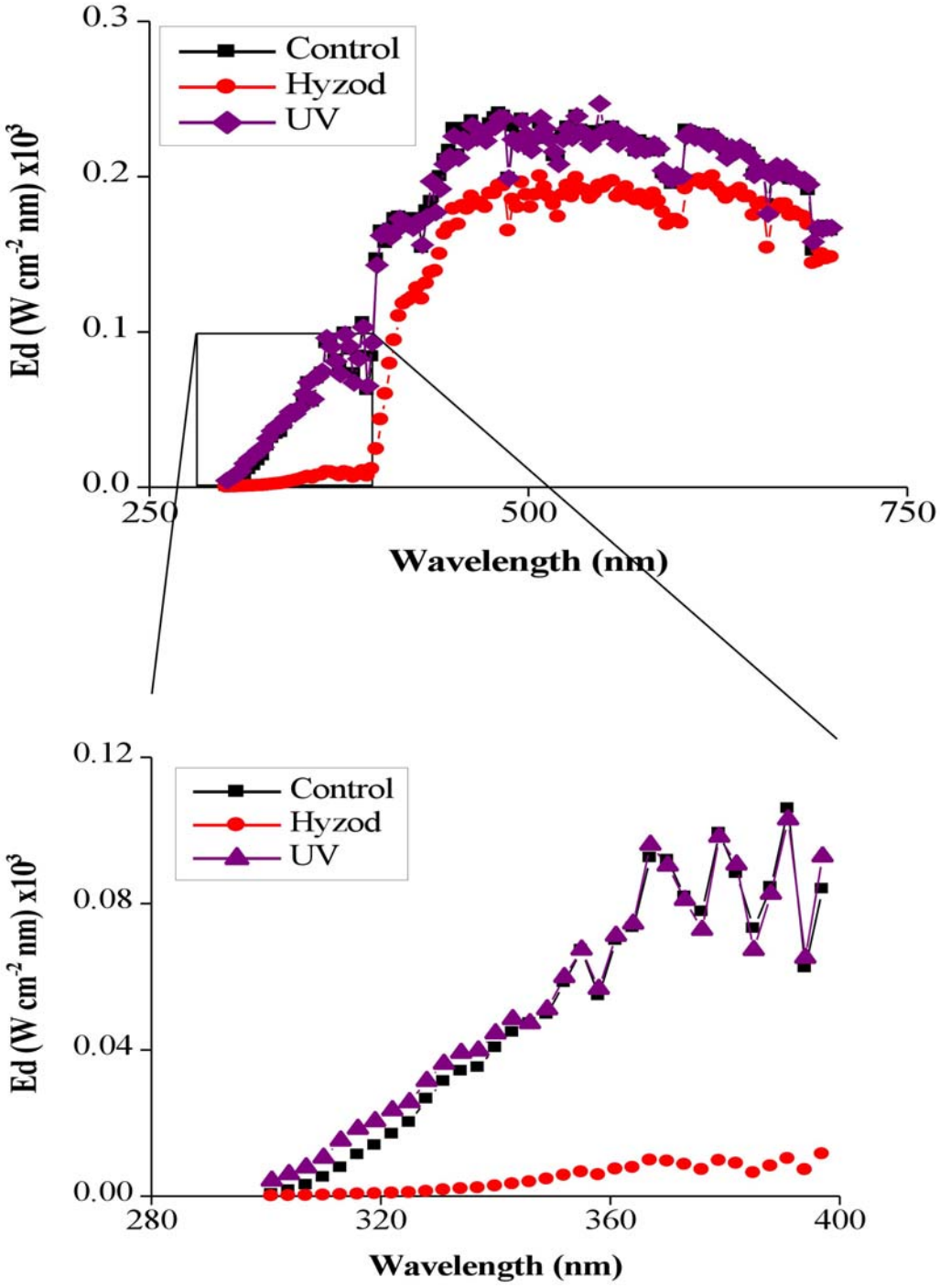


Figure 15. Downwelling irradiance (Ed) measured with the Optronics OL-754 spectroradiometer in the outdoor aquariums at Magueyes Island at the end of the UV-S Experiment (July 29, 2003).

**Other physical parameters measured during both experiments**

A HOBO® temperature logger that recorded water temperature each hour was located at San Cristóbal Reef at the control and one of each of the treatments during the UV-E Experiment. During the UV-S Experiment, similar loggers were located at San Cristóbal Reef at 1m and at 20m at the Old Buoy site at the shelf edge. Three temperature loggers were also located at the outdoor aquariums during the UV-S Experiment (one per treatment, including the controls). Hence, the temperature records obtained at San Cristóbal during the UV-S Experiments were also used as references to the measurements taken at the aquariums. The data were recovered at monthly intervals. A One-Way ANOVA was used to test for differences among temperatures recorded in both the UV-E and UV-S Experiments.

Temperature was recorded at one hour intervals during both experiments (Table 5). No significant differences were found among temperature means (ANOVA, $p=0.621$), minima (ANOVA, $p=0.485$), or maxima (ANOVA, $p=0.693$) recorded in both the UV-E and UV-S Experiments. Interestingly, temperature minima were recorded daily at night (02:00-03:00), and maxima around 10:00-14:00; similar to UVR levels during the day.

Table 5. Temperature measurements during the UV-E (March-July, 2001) and UV-S Experiments (March-July, 2003). Numbers for average temperature represent the average ± 1SD.

| Location | Minimum Temp (°C) | Maximum Temp (°C) | Average Temperature (°C ± 1SD) |
|---|---|---|---|
| **UV-E Experiment:** | | | |
| Control | 24.8 | 27.9 | 26.5 ± 0.6 |
| Hyzod | 24.4 | 27.5 | 26.1 ± 0.5 |
| Saran | 24.3 | 27.6 | 25.9 ± 0.4 |
| **UV-S Experiment:** | | | |
| *Field:* | | | |
| San Cristóbal Reef (1m) | 24.9 | 28.3 | 26.1 ± 0.3 |
| Old Buoy shelf edge site (20m) | 23.8 | 27.7 | 25.4 ± 0.2 |
| *Aquariums:* | | | |
| Control | 25.2 | 28.3 | 27.0 ± 0.6 |
| Hyzod | 24.8 | 28.0 | 26.3 ± 0.2 |
| UV | 25.0 | 28.4 | 26.9 ± 0.3 |

## SUMMARY

When examining the effects of incident UVR on living organisms it is important to be able to modify the amount of radiation they receive and accurately measure these. This process was explored in the present study in two different ways: 1) by excluding more than 98% of the incident UVR at 1.5m depth at a nearshore reef in La Parguera, Puerto Rico, and 2) by enhancing the amount of UVR received by the experimental coral colonies either naturally or artificially.

In the UV-E Experiment, in order to obtain a relatively quick organismal response from the studied corals, the colonies were exposed to more than 98% depleted UVR levels and 23 and 82% of the incident PAR levels under a Hyzod® acrylic panel and a Saran® mesh, respectively. Alternatively, the levels of UVR and PAR reaching the clear shallow-waters of the back-reef area at San Cristóbal Reef in La Parguera were also measured. An approximate 62% in PAR attenuation was found reflecting the high

influence of scattering from the presence of particulate and dissolved matter even in these relatively clear waters.

$Kd_{UVR}$ and $Kd_{PAR}$ values were higher in nearshore reef waters compared to shelf edge waters. The varying patterns observed in $Kd_{PAR}$ and $Kd_{UVR}$ also indicate the importance of presence/absence of DOM and particulate matter, absorption by the water molecules, and concentration of phytoplankton, among others (Kirk, 1984), in controlling the amount of UVR and PAR reaching coral reef waters.

The enhanced UVR doses used during the UV-S Experiment in the outdoor aquariums were similar to those used before in similar experiments performed with phytoplankton (Cullen and Lesser, 1991). The results were also similar to those of Shick et al. (1999) who exposed colonies of *Stylophora pistillata* to enhanced UVR levels using a similar setup, but with Q-Panel 340nm UV fluorescent lamps. Nonetheless, the usage of similar lamps but with a peak emission in the UV-B range (Q-Panel 313nm) provides a more appropriate setup for studying effects of UVR in marine organisms, especially considering the more deleterious effects of short-wavelength energy (UV-B) in terrestrial and marine organisms (Worrest et al., 1978; Diffey, 1991).

Other factors that may influence the physiological responses of reef corals such as temperature increases were also measured. The bleaching observed in *Acropora cervicornis* during the UV-S Experiment (see Chapter 3) was not related to the normal temperature fluctuations seen at San Cristóbal Reef. Similar temperatures were observed at both study sites (San Cristóbal and the Old Buoy) throughout the experiment despite the difference in depth. Therefore, the bleaching observed in *A. cervicornis* colonies

transplanted from deep to shallow waters was produced as a result of changes in the quality and/or quantity of radiation received by the colonies.

# CHAPTER I: EFFECTS OF CHANGES IN UV RADIATION ON THE GROWTH OF *ACROPORA CERVICORNIS* AND *PORITES FURCATA*

## ABSTRACT

The linear extension rates and skeletal density of *Acropora cervicornis* and *Porites furcata* were examined under reduced incident ultraviolet radiation (UVR; 280-400nm) at La Parguera, Puerto Rico. Thirty-six colonies of each species were exposed to depleted UVR and reduced PAR under Hyzod® acrylic panels or Saran® meshes (18 colonies/species/treatment) at San Cristóbal Reef. Linear extension rates of both species were significantly higher (Fully Nested ANOVA, $p<0.0001$) after being exposed for 88 days to 1% UVR compared to controls (Hyzod®). Yet, linear extension rates were also significantly reduced after being exposed to reduced PAR levels (Saran®). There were no significant differences in skeletal density of *A. cervicornis* ($p=0.313$) among treatments. The reduction in the skeletal density of *P. furcata* in both treatments and controls, while not significant (Fully Nested ANOVA, $p=0.132$) suggests the influence of a species-specific factor. Another experiment examined the effects of enhanced UVR in both species by a) transplanting ten colonies of *A. cervicornis* from the shelf edge (20m) and ten colonies from El Palo Reef (8m) to the back-reef area of San Cristóbal Reef (1m) and vice versa, and b) transplanting nine colonies of *P. furcata* to outdoor aquariums with Q-Panel 313nm UV lamps. Another nine colonies of *P. furcata* were transplanted to running seawater aquaria shielded with a Hyzod® acrylic panel and nine additional colonies were

transplanted and exposed to mimicked UVR and PAR levels (controls). All the colonies of *P. furcata* were collected at San Cristóbal Reef (1m depth). Both species showed significantly reduced linear extension rates when exposed to enhanced UVR, except those of *A. cervicornis* transplanted from 8m to 1m, which grew faster, but developed less dense skeletons than those transplanted to deeper areas. The linear extension rates in *A. cervicornis* were reduced 66% in colonies transplanted from 20 to 1m, probably due to intense bleaching a few hours after the transplant. *Porites furcata* colonies exposed to enhanced UVR showed a 25% decrease in linear extension rates and less dense skeletons compared to controls. These results support the hypothesis that deleterious effects may occur to shallow-water reef-building corals with an increase in UVR.

## INTRODUCTION

The depletion of the Earth's ozone layer and the consequent increases in ultraviolet radiation (UVR) levels reaching the Earth has captured the attention of many scientists and the general public during the last decade. In tropical marine environments, UVR absorption may fluctuate between 20-50% per meter depth, even though in some coastal areas it can reach up to 90% (Smith and Baker, 1979). In clear waters UV can penetrate up to 20m depth (Fleischmann, 1989). Coral reefs are especially sensitive to increases in UVR due to the high transparency of the oligotrophic waters were they live (Calkins and Thordardottir, 1980; Diffey, 1991; Gleason and Wellington, 1993; Bijlsma et al., 1995; Häder et al., 2003).

The effects of UVR on reef-building corals has received widespread attention in the Pacific Ocean during the last decade (i.e., Drollet et al., 1993; Kinzie, 1993; Krupp and Blanck, 1995; Lewis, 1995; Lesser and Lewis, 1996). Nevertheless, comparatively less literature exists on the response of Caribbean corals to this environmental physical factor (Gleason, 1993; Gleason and Wellington, 1993, 1995; Muszynski, 1997; Muszynski et al., 1998; Corredor et al., 2000).

So far, no study has quantified the effects of incident levels of UVR on shallow-water Caribbean branching corals. There are, however, some reports on the effects of UVR on the coral host physiology and the correspondent dinoflagellate symbionts (i.e., inhibition of photosynthesis; changes in the zooxanthellae concentration; lower algal mitotic indices; lower linear skeletal extensions; among others) on some Caribbean massive coral species (Gleason, 1993; Gleason and Wellington, 1995).

The present chapter presents direct evidence of the detrimental effects of actual and enhanced levels of UVR on Caribbean shallow-water branching corals. It compares the growth rates, in terms of linear extension rates and skeletal density, of the Caribbean shallow-water branching corals *Acropora cervicornis* (Lamarck 1816) and *Porites furcata* (Lamarck 1816) under different Photosynthetically Active Radiation (PAR) and UVR regimes.

# METHODS

## Growth analysis

If a coral is transplanted to an area where it is only rarely found and if adequate and frequent growth measurements are made, then the growth rate can be used as an index of the coral's adaptation to the environment (Shinn, 1966). The skeletal growth of both species (by means of linear extension rates and skeletal density) was used in this study as one of the main physiological parameters measured.

Before locating the colonies haphazardly at any particular treatment, they were stained with Alizarin Red S at a concentration of 15mg $l^{-1}$ for 8 hours at the collection site (Lamberts, 1978) and were let to rest for five days. Afterwards, the colonies were checked for any signs of stress as a result of the staining.

Two growth parameters were used during both experiments: linear extension rates and skeletal density. To measure linear extension rates (as a measure of the increase in length in mm along the growth axis, *sensu* Gladfelter, 1984), the branches were put in a 5% sodium hypochlorite solution for 24 hrs for tissue digestion, washed with fresh water and the growth above the stain line was measured with a Vernier caliper to the nearest 0.1mm. Measurements were taken from the stain line to the tip of the branch; when more than one branch was used, averages for the colony were recorded. Linear extension rates were estimated dividing the skeletal extension (above the stain line) by the days of exposure.

Staining was not performed in *A. cervicornis* during the UV-S Experiment. Instead, a plastic tie was attached to the main branch of each colony to measure the linear

extension rates at the end of the experiment. The length from the plastic tie to the tip of the branch was measured on-site with a Vernier caliper to the nearest 0.1mm at the beginning of the experiment and used as reference for subsequent measurements. Linear extension rates of *P. furcata* during the UV-S Experiment were measured similarly to the UV-E Experiment (Alizarin Red staining technique).

Skeletal density for both UV-E and UV-S Experiments was measured using the liquid displacement technique. The tissue-free piece of each colony used for the pigment analysis (see Chapter III below) was left to dry for a week at room temperature and weighed to the nearest 0.0001g in an analytical balance. Then, quickly submerged in a test tube and the volume recorded to the nearest 0.1ml. The density was obtained dividing the weight by the volume.

**Statistical analysis**

Data was tested for normality and equality of variances using a Bartlett's Test with Bonferroni 95% confidence intervals. Data was log-transformed whenever unequal variances were found to comply with the statistical testing assumptions. Based on the experimental design, a Fully Nested ANOVA was used to test for statistical differences in linear extension rates and skeletal density between colonies among replicates of the same treatment, between replicates of the same treatment, and among treatments. Where statistical differences were found, a Tukey test with Pairwise Comparisons was performed to distinguish where these differences were (Ott and Longnecker, 2001). Statistical significance was set at $\alpha = 0.05$.

# RESULTS

## UV-Exclusion Experiment

The linear extension rates (mm $d^{-1}$) of *Acropora cervicornis* were significantly higher in colonies growing under the Hyzod® panels compared to those of colonies growing at ambient levels of UVR (control colonies) (0.46 ± 0.02, 0.39 ± 0.02, respectively; Fully Nested ANOVA, $p<<0.001$; Fig. 1.1) during the UV-E Experiment. This represents an increase in growth of 14-22% for this species under no UVR conditions. A significant decrease in linear extension rates between 18-42% was found in those colonies living under the Saran® mesh compared to the controls and those growing under the Hyzod® (Tukey test: $p=0.006$, $p<0.001$, respectively), where PAR levels were considerably lower than the normal levels at the experimental depth, suggesting an interaction effect of both types of radiation on the growth rates of the species. No differences were found in the linear extension rates of *A. cervicornis* between colonies within replicates of the same treatment and between replicates within treatments (Fully Nested ANOVA, $p=0.489$).

In the case of *Porites furcata*, similar results were found. Linear extension rates (mm $d^{-1}$) were higher in colonies living under no UVR, but reduced growth was found when PAR levels were also reduced (control colonies, 0.14 ± 0.013; Hyzod® 0.16 ± 0.003; and Saran® 0.13 ± 0.018; Fully Nested ANOVA, $p<0.01$; Fig. 1.1). The former represents an increase in growth of *P. furcata* between 6-18% under no UVR. A Tukey test showed no significant differences between the control and Saran® treatment ($p=0.576$). No significant differences were found in the linear extension rates of *P.*

*furcata* between colonies among replicates of the same treatment or between replicates of the same treatment (Fully Nested ANOVA, p=0.811). The relationship between linear extension rates and depleted UVR levels for both species is shown in Figure 1.2. Linear extension rates of both species were significantly higher under 99% depleted UVR compared to controls exposed to actual UVR levels (t-test: p=0.007 for *A. cervicornis* and p=0.002 for *P. furcata*).

No significant effect was found in the skeletal density of the *A. cervicornis* colonies between treatments (Fully Nested ANOVA, p=0.732; Fig. 1.3). In fact, no differences were found in the skeletal density of *A. cervicornis* colonies exposed to normal vs. 99% depleted UVR conditions (Figure 1.4; t-test, p=0.788). No differences were found in the skeletal density of *A. cervicornis* between colonies within replicates of the same treatment and between replicates within treatments (Fully Nested ANOVA, p=0.641). While not significant, the skeletal density of *P. furcata* was reduced in all treatments including the controls (Fully Nested ANOVA, p=0.132; Fig. 1.3) compared to the skeletal density measured at the beginning of the experiment. Yet, a comparison between the skeletal density of *P. furcata* living at normal UVR levels vs. depleted UVR and PAR levels did showed a significant relationship (Figure 1.4; t-test, p=0.003). No significant differences were found in the skeletal density of *P. furcata* between colonies among replicates of the same treatment or between replicates of the same treatment (Fully Nested ANOVA, p=0.566).

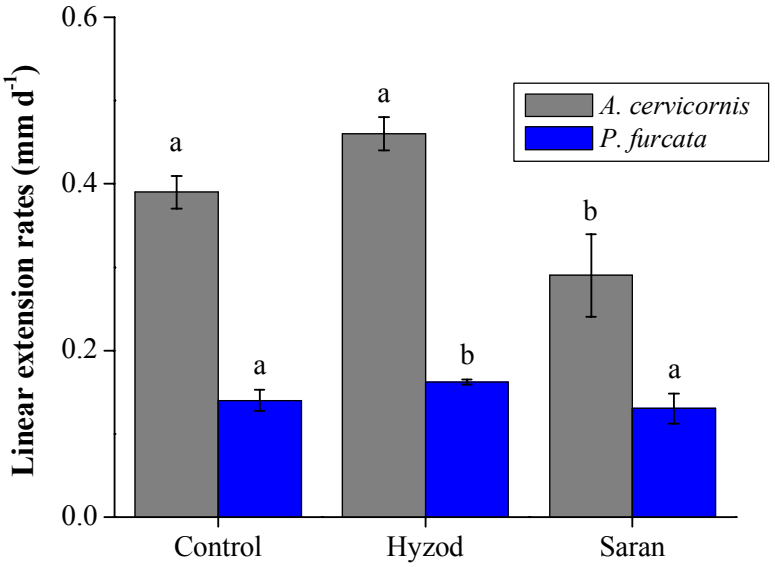


Figure 1.1. Average linear extension rates of both species measured after 88 days of exposure under the three different treatments. Vertical lines represent ± 1SD. Different letters represent significantly different data (α = 0.05).

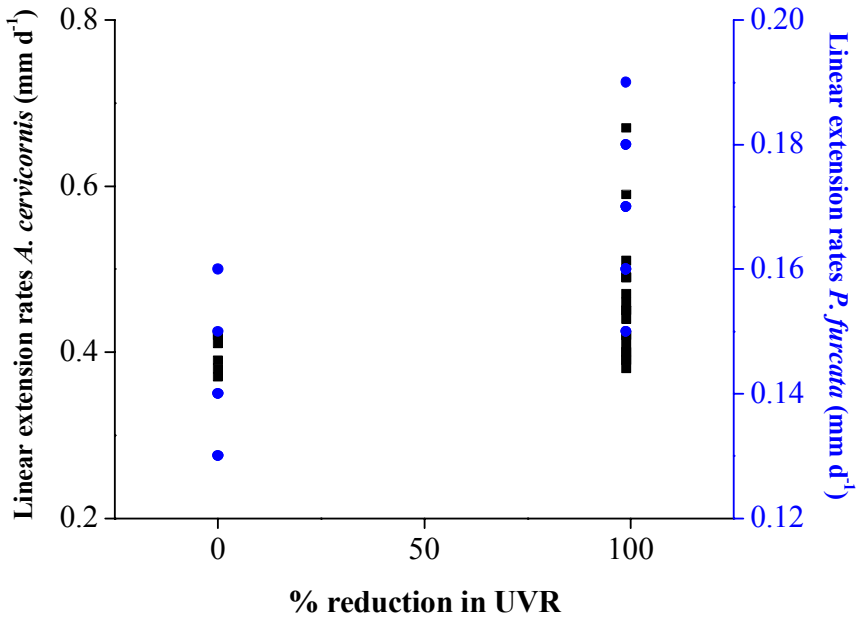


Figure 1.2. Relationship between the reduction in UVR levels and the linear extension rates of both species.

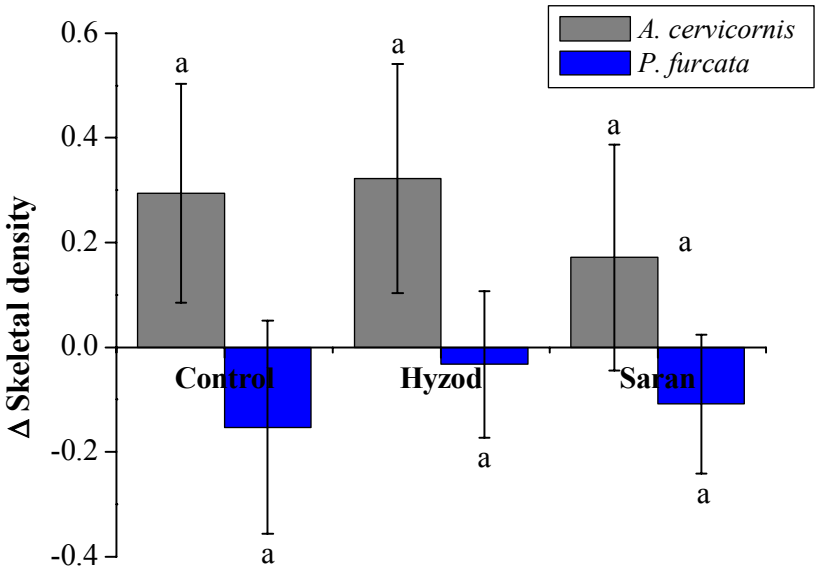


Figure 1.3. Change in skeletal density in both species after 88 days of exposure under the two different treatments compared to controls. Δ skeletal density = skeletal density at the end of the experiment – skeletal density measured at the beginning of the experiment. Vertical lines represent ± 1SD. Different letters represent significantly different data (α = 0.05).

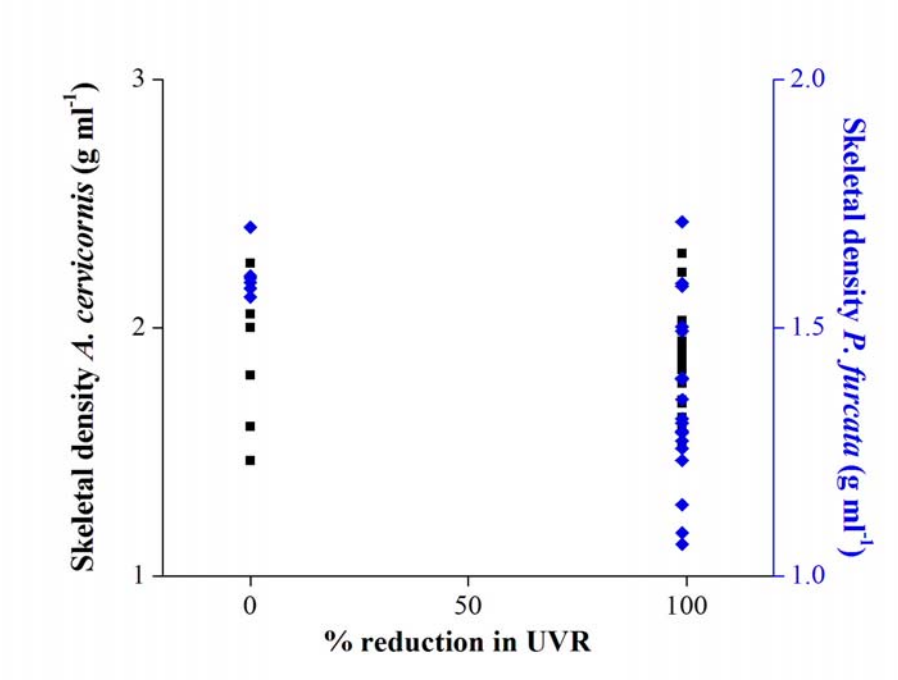


Figure 1.4. Graph showing interspecific differences in changes in skeletal density with depleted UVR.

**UV-Supplementation Experiment**

Significant differences were found in the linear extension rates of those colonies transplanted to different depths vs. the controls at those depths (Fully Nested ANOVA, $p<0.0001$). The colonies of *A. cervicornis* grew significantly less when transplanted from 20m to 1m depth compared to the control colonies at 20m ($0.30 \pm 0.004$ vs. $0.83 \pm 0.0004$, respectively; Tukey Test, $p<0.0001$). This was accompanied by bleaching in these colonies that began one day after the transplant and continued for more than 90 days (see Chapter III). Colonies transplanted from 8m to 1m depth had significantly higher linear extension rates than the rest of the colonies including the control colonies at 1m (Tukey Test, $p<0.01$, Figure 1.5). However, a significantly less dense skeleton was found in the colonies transplanted from 8m to 1m compared to the control colonies at 1m (Tukey Test, $p<0.001$, Figure 1.6). No significant differences were found among individual colonies of the same treatment (Fully Nested ANOVA, $p=1.000$).

While there was no significant change in the linear extension rates of colonies transplanted from shallow (1m) to deep (20m) waters (Tukey Test, $p=0.196$) compared to the controls at 1m, these colonies showed the greatest positive variation in skeletal density compared to the control and the other transplanted colonies (Tukey test, $p<0.001$). No significant differences were found in the skeletal density among colonies left on-site as controls at San Cristóbal and the Old Buoy shelf edge site or among colonies transplanted from one specific site to another (i.e., among those colonies transplanted from a particular depth) (Fully Nested ANOVA, $p=0.990$).

In the case of *P. furcata*, there was a significant decrease in linear extension rates (Fully Nested ANOVA, $p<<0.0001$, Figure 1.7) in colonies exposed to increased levels

of UVR, and in the skeletal density (Fully Nested ANOVA, $p<0.001$, Figure 1.8). Those colonies living under the Hyzod® panels also exhibited significantly greater linear extension rates than the controls (Tukey Test, $p=0.022$) accompanied by similar increases in skeletal density (Tukey Test, $p=0.001$). There were no significant differences in linear extension rates between colonies among replicates of the same treatment or between replicates of the same treatment (Fully Nested ANOVA, $p=0.415$). Appendix I shows a summary of the statistical results of the measurements of linear extension rates and skeletal density for *A. cervicornis* and *P. furcata* for both UV-E and UV-S Experiments.

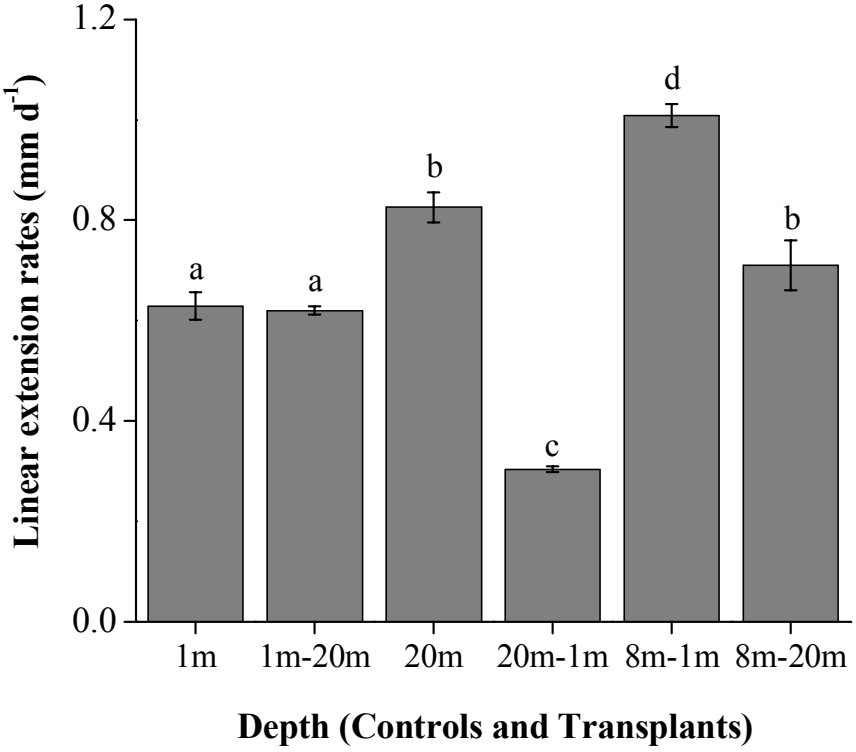


Figure 1.5. Average linear extension rates of *A. cervicornis* 115 days after transplanting the colonies to the different sites. Legend: 1m = control colonies at the back-reef area of San Cristóbal Reef; 20m = control colonies at the Old Buoy shelf edge area; other columns refer to original vs. transplanted depth. No controls were available at 8m since colonies left at El Palo Reef were not found after the original transplantation. Vertical lines represent ± 1SD. Different letters represent significantly different data ($\alpha = 0.05$).

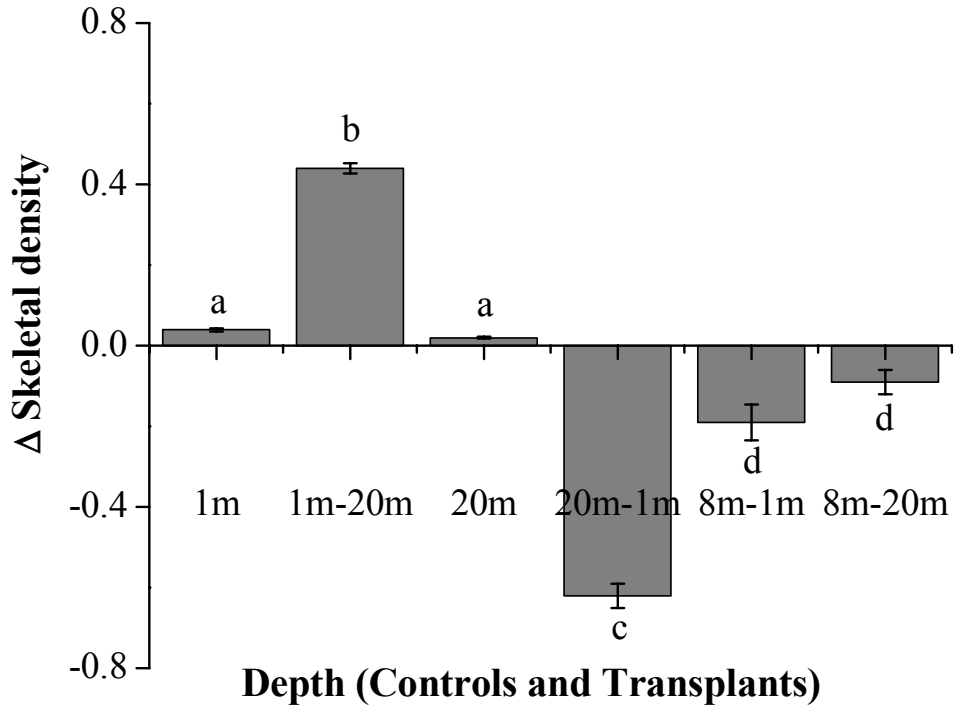


Figure 1.6. Change in skeletal density of *A. cervicornis* 115 days after transplanting the colonies to the different sites. 1m = control colonies at the back-reef area of San Cristóbal Reef; 20m = control colonies at the Old Buoy shelf edge area; other columns refer to original vs. transplanted depth. Vertical lines represent ± 1SD. Different letters represent significantly different data ($\alpha = 0.05$).

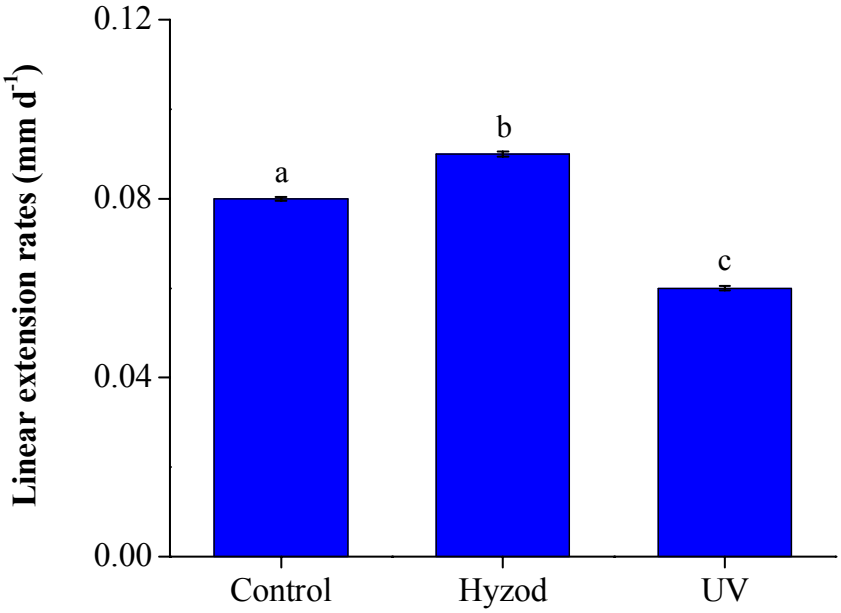


Figure 1.7. Average linear extension rates of *P. furcata* after 128 days of exposure to the different treatments in outdoor aquariums at Magueyes Island. Vertical lines represent ± 1SD. Different letters represent significantly different data (α = 0.05).

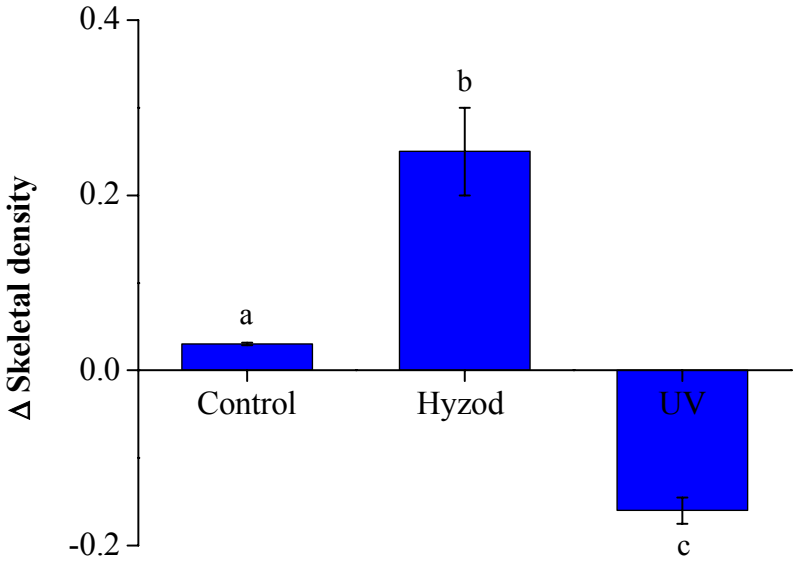


Figure 1.8. Change in skeletal density of *P. furcata* after being exposed to the different treatments for 128 days in outdoor aquariums at Magueyes Island. Vertical lines represent ± 1SD. Different letters represent significantly different data (α = 0.05).

# DISCUSSION

Solar UVR penetrates to biologically significant depths in natural waters, although the degree of penetration, particularly in the UVB region, strongly depends on the spectral absorption by the dissolved and suspended material in the water column (Baker et al., 1980; Diffey, 1991). This is particularly important in clear tropical reef waters where UVR can penetrate up to 20m depth (Fleischmann, 1989). It is in these clear waters where reef corals thrive and grow to their maximum potential (Barnes, 1973; Battey and Porter, 1988; Barnes and Chalker, 1990; Villinski, 2003).

Coral growth can be affected by numerous environmental factors including sedimentation (Rogers, 1990; Torres, 2001; Torres and Morelock, 2002), eutrophication (Tomascik and Sanders, 1985), and solar UVR (Gleason, 1993; this study). Edmunds and Davies (1989) found reduced energy investments in growth in stress colonies of *Porites porites* at Discovery Bay, Jamaica. In the present experiment, significantly lower linear extension rates were found in *Acropora cervicornis* and *Porites furcata* in colonies exposed to normal levels of PAR and UVR compared to those of colonies under 1% UVR. Both species showed even significantly lower linear extension rates when 82% of the normal PAR levels at 1.5m depth at San Cristóbal Reef were reduced under the Saran® mesh. This corroborates the importance of the longer visible wavelengths in coral growth and metabolism (Falkowski et al., 1990). Gleason (1993) found similar results [to those presented here] in the growth of transplanted colonies of the brown morphs of *Porites astreoides* in St Croix, USVI and attributed his results in part to the high metabolic cost of producing large quantities of mycosporine-like amino acids (MAA's) to

block UVR. He also found reduced concentrations of zooxanthellae in corals exposed to high levels of UVR compared to those shaded from UVR. The present results support Gleason's (1993) findings, since, even though the zooxanthellae concentrations in colonies of both *A. cervicornis* and *P. furcata* were not significantly different among treatments, a trend of higher concentrations of zooxanthellae in the UVR-shaded colonies was observed (see Chapter III). Furthermore, MAA's concentrations were significantly increased in colonies of *P. furcata* and *A. cervicornis* exposed either artificially or in the field to enhanced levels of UVR. This study presents evidence on the deleterious effects that a minimum increase in UVR may cause to the formation of the corals exoskeleton in terms of the reduction of the coral's linear extension rates and the production of less dense skeletons when to higher UVR levels than those that penetrate to the depths at which these corals exist.

Decreased skeletal densities in all colonies of *P. furcata* at all treatments during the UV-E Experiment suggest a species-specific factor influencing this characteristic. The results might reflect an annual cycle affecting the deposition of aragonite in part of the skeleton. This was not observed in *A. cervicornis* supporting the hypothesis that inherent species-specific factors, independent of the zooxanthellae photosynthesis, also exert an important influence on the skeletal $CaCO_3$ deposition (Goreau, 1961). For instance, while *A. cervicornis* colonies calcify at a faster rate than *P. furcata*, the skeletons appear to be denser in the former than those of the latter. These differences in the calcification process among both species might have accounted for the marked differences in skeletal density found during the UV-E Experiment.

During the UV-S Experiment, all colonies of *A. cervicornis* transplanted from 20m to 1m depth showed signs of bleaching in the upper part of their branches one day after being transplanted. This bleaching was considered an effect of the drastic change in light levels and not of the transplantation event since only the colonies transplanted from 20m to 1m bleached. The other colonies transplanted from 8m to 1m or from 1m to 8m and 20m did not show any signs of bleaching after transplantation. Nonetheless, the colonies did not die throughout the experiment. In fact, at the end of the experiment they seem to be adapting to enhanced UVR and PAR levels as they were in the process of regaining their normal coloration (see Chapter III). Yet, the linear extension rates of these colonies were significantly reduced compared all other transplants (see Figure 1.5 above) similar to the growth of *Montastraea annularis* colonies after the 1987-bleaching event in Jamaica and Florida (Goreau and Macfarlane, 1990; Szmant and Gassman, 1990; Leder, et al., 1991).

Coral skeleton accretion during a bleaching event can even stop (Leder et al., 1991) and results from *M. annularis* suggest that calcification, more than photosynthesis, is inhibited during bleaching events (Goreau and Macfarlane, 1990). Szmant and Gassman (1990) found a 30% decrease in coral tissue carbon and 44% decrease in coral tissue nitrogen biomass per skeletal surface area in bleached colonies. Similarly, Leder et al. (1991) found a 63% reduction in mean annual growth in *M. annularis* after the mass bleaching event of 1987 in Florida. The bleached *A. cervicornis* colonies that were transplanted from the shelf edge to San Cristóbal Reef may have survived by, among other ways, re-absorbing their reproductive structures (see Szmant and Gassman, 1990),

since these were not seen anymore after the transplantation and bleaching event of thee colonies (see also Chapter II).

The significant reduction in linear extension rates of both species at incident levels of PAR and particularly UVR found in the present study presents evidence on the detrimental effects that actual and expected increased levels of UVR may cause to the skeletal growth of shallow-water scleractinian corals. Growth rates (e.g. linear extension rates and skeletal density) of both species were negatively correlated with normal levels of UVR and enhanced UVR daily doses. This may be coupled with the expected increase in global warming-related factors such as high $CO_2$ levels and the decrease in the aragonite saturation state in the ocean (Orr et al., 2005; Pelejero et al., 2005).

Considering that coral reef calcification is predicted to decrease 20-60% by 2100, relative to pre-industrial levels (Kleypas et al., 1999; Marubini et al., 2003; Reynaud et al., 2003; Müller et al., 2004; Orr et al., 2005; Pelejero et al., 2005) and that increased UVR may induce the formation of a less denser skeleton and reduced linear extension rates, the scenario for reef corals is uncertain. However, the impact of increases in UVR on marine shallow-water calcifying organisms, such as scleractinian corals, is unclear. Nonetheless, it may depend on the ability of the organisms to withstand future physical and environmental conditions. The present results suggest that further increases of UVR as a result of the ozone layer thinning could have detrimental effects on shallow water branching corals.

# CHAPTER II: EFFECTS OF CHANGES IN UV RADIATION ON THE FECUNDITY OF *ACROPORA CERVICORNIS* AND *PORITES FURCATA*

## ABSTRACT

The effects of enhanced UVR on the fecundity of *Acropora cervicornis* were measured in field-transplanted colonies from 20 and 8m to 1m depth at La Parguera, Puerto Rico. Fecundity was estimated from histological sections made from tissue samples taken at the beginning, middle, and end of the experiment. Colonies transplanted from 20m to 1m depth showed a 100% reduction in fecundity (i.e., gonads per mesenteries per polyp) due to reabsorption of abortion of gonads, while those transplanted from 1m and 8m to 20m depth did not show any significant reduction in fecundity. These colonies did show however, a delay in the spawning times by releasing their gamete bundles approximately two-three weeks after the controls at 1m and one month after the controls at 20m as a response to changes in their daily light cycle due to less radiation (PAR and UVR) available at 20m compared to 1m. Transplants from 8m to 1m spawned after the full moon of July 29, 2003 similarly to controls at 1m. Results suggest a possible strategy for enhancing reproductive success at 1m. The effects of enhanced UVR on the fecundity of *Porites furcata* were measured by exposing nine colonies collected at 1m depth at San Cristóbal Reef to artificially enhanced UVR in outdoor aquariums. Another nine colonies was exposed to reduced UVR levels by placing a Hyzod® acrylic panel over the aquariums. Nine additional colonies were

transplanted and exposed to normal UVR and PAR levels (controls). The same histological techniques were used for this species. A significant reduction was found in the number of spermaries per mesentery through out the experiment in those colonies exposed to enhanced UVR. A similar reduction was observed in the number of eggs per mesentery, but only after three months of exposure. All the colonies of *P. furcata* exposed to reduced UVR showed fully developed eggs and spermaries during the three sampling dates. While a possible reabsorption of the gametes occurred in *A. cervicornis* colonies transplanted from 20m to 1m, the expulsion of these gametes due to the sudden stress caused by the transplantation is not discarded.

## INTRODUCTION

The majority of reef corals are broadcasters, this is, they release their gametes into the water column where they eventually fuse to form a planulae larva (Krupp, 1983; Szmant, 1986; Richmond and Hunter, 1990; Soong, 1991; Steiner, 1995). Nonetheless, a limited number of scleractinian corals are known to release already developed larvae directly from the polyp mouth (brooders) (Fadlallah, 1983; Chornesky and Peters, 1987; Harrison and Wallace, 1990; Soong, 1991).

Several studies have already been done on the effects of UVR on the gametes of aquatic organisms; the majority of these are associated with fishes (Dey and Damkaer, 1990; Grunwald and Streisinger, 1992). These studies showed a sterilizing effect on the eggs given high doses of UVR. When sperm were irradiated at low doses and then used to fertilize normal eggs, survival was very low. This was apparently caused from the

inactivation of the sperm's chromatin. Similarly, in reef corals, Gulko (1995) made cross-fertilization using sperms (previously exposed to UV-B) and eggs of the Pacific coral *Fungia scutaria* and found a significant decrease in sperm motility and viable planulae compared to planulae developed from unexposed gametes.

Even though one reproductive cycle might be insufficient to produce a detectable difference in tolerance (i.e., acclimation) to UVR, and that longer acclimation periods are required to demonstrate an effect (Baker, 1995), the present study was done during the peak periods of gametogenesis for both species (Soong, 1991). The corals were exposed to their respective experimental setups from March to July 2003. In such way, the effects of increasing and decreasing the intensity of the UVR received by the colonies could be measured during the months of gonad development (eggs and sperm) and eventual spawning or larvae release. While fecundity can be used as a stress indicator in reef corals (Harrison and Wallace, 1990; Michalek-Warner and Willis, 2001a), the effects of sub-lethal impacts on coral reproduction are not easily separated from natural variations due to factors such as size or age (Szmant, 1991). This factor was avoided during this study by selecting similarly-sized colonies of each species.

The objective of this chapter is to study the effects of enhanced UVR on the fecundity of both species. The null hypothesis states that reproductive effort (measured as the number of eggs/bundle in the case of the broadcasting species *A. cervicornis*; and number of eggs and released larvae per polyp in the case of the brooding species *P. furcata*), will not be affected by changes in UVR. The alternate hypothesis ($H_1$) states that a significant reduction in the fecundity will be observed under enhanced UVR and an increase will occur under reduced UVR.

# METHODS

Coral fecundity was determined following a histological protocol similar to that described by Vargas-Toledo (2002). A section from the proximal end of each sampled branch was used (Figure 2.1). The use of fragments from any other part of the branches was avoided since usually the distal polyps of branching corals are more dedicated to colony growth and do not contain any reproductive structures (Oliver, 1984; Szmant, 1986; Soong, 1991). The tissue was fixed using a 10% Formalin in seawater solution for 24hrs and rinsed in deionized water (DIW) for another 24hrs to eliminate excess of fixing solution. Then, the coral piece was decalcified using a 10%HCL 0.7%EDTA solution. The tissue was cleaned, put into labeled embedding capsules and soaked into a 75% Ethanol solution until the histological procedure.

After tissue fixing and preservation, samples were taken to the Puerto Rico Department of Natural and Environmental Resources, Marine Fisheries Laboratory for histological procedures. Coral tissues were dehydrated and cleared using a Tissue-Tek Vacuum Infiltration Processor (VIP 1000, Model 4617). Samples were dehydrated in a series of stations as follows: 70%EtOH (twice), 95%EtOH, 100% Isopropanol (three times), Xylene (three times), and Paraffin (twice). Samples were kept in each station for 1hr. Then, the tissues were embedded both transversally and longitudinally using liquid paraffin, solid-dry, and kept refrigerated at $4^{o}$C until sectioning. Sectioning was performed in a Reichert-Jung 820-II microtome at 7µm. Three to five slides were obtained from each sample. Care was taken to produce slides that contain tissue from the middle and basal part of the polyps in both longitudinal and transversal sections. Samples

were stained using the Heidenhain's Aniline-Blue method, which consisted in soaking the tissue slides in a series of stations as follows: Xylene I, II, III, 100%EtOh, 100%EtOH, 100%EtOH, 95%EtOH, 70%EtOH, DIW, Azocarmine B at 56ºC, tap water, DIW, Aniline-Alcohol, DIW, Phosphotungstic Acid, DIW, Aniline-Blue, tap water, DIW, 70%EtOH, 95%EtOH, 100%EtOH, 100%EtOH, 100%EtOH, and Xylene IV, V, VI. The slides were covered with a cover glass and let dry for 2 days.

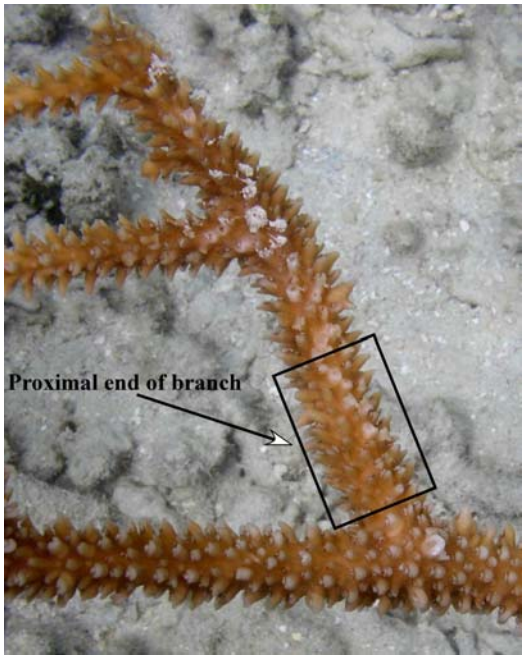


Figure 2.1 An example of an *A. cervicornis* branch with an approximation of the section used for histolgical analysis.

Gonads were identified using a compound microscope at 100x. The maturation stage of eggs and spermaries present, number of each by mesenteries, number of mesenteries containing gonads per polyp, and average number of gonads per polyp was noted following previously established criteria (Szmant-Froelich et al., 1985; Chornesky and Peters, 1987; Soong, 1991). Also, the percentage of polyps containing gonads (either

eggs or spermaries) was obtained by analyzing ten haphazardly chosen polyps within the tissue sample. Data of all the colonies within a treatment were pooled for later statistical analysis. Three additional colonies of each species spread ten meters apart from each other were haphazardly chosen at San Cristóbal Reef at 1m depth. Histological methodology was performed as above. These colonies were used as a reference when examining the fecundity results of control and treated colonies of both species during the UV-S Experiment.

Based on the experimental design, a Fully Nested ANOVA was used to test for statistical differences in fecundity between colonies among replicates of the same treatment, between replicates of the same treatment, and among treatments in both species. Where statistical differences were found, a Tukey test with Pairwise Comparisons was performed to distinguish where these differences were (Ott and Longnecker, 2001). Statistical significance was set at $\alpha = 0.05$.

Due to technical problems with the tissue fixation during the UV-E Experiment, only the data from the UV-S Experiment was analyzed. Nonetheless, it is sufficient to show the potential deleterious effects an increase in present levels of UVR might have in the fecundity of shallow-water reef corals.

# RESULTS

The *Acropora cervicornis* colonies transplanted to deeper areas (i.e., El Palo Reef and San Cristóbal Reef to the Old Buoy site at the shelf edge) spawned in the aquaria on August 18 (six days after the full moon of August 12, 2003). Those colonies transplanted from San Cristóbal Reef to the shelf edge (Old Buoy) suffered a delay in the spawning time of approximately two-three weeks, hence next lunar cycle, compared to the control colonies at 1m depth at San Cristóbal Reef which spawned after the new moon of July 29, 2003, and approximately one month compared to the control colonies at 20m depth at the Old Buoy shelf edge site as can be inferred from Figure 2.2. A sample photo of the eggs/sperm bundles released by the colonies transplanted from El Palo Reef and San Cristóbal Reef to the Old Buoy is shown in Figure 2.3. The gamete bundles from these colonies contained an average of $5 \pm 0.8$ and $4.3 \pm 1.1$ eggs/bundle, respectively. All the released bundles from these colonies contained one sperm package (Figure 2.3).

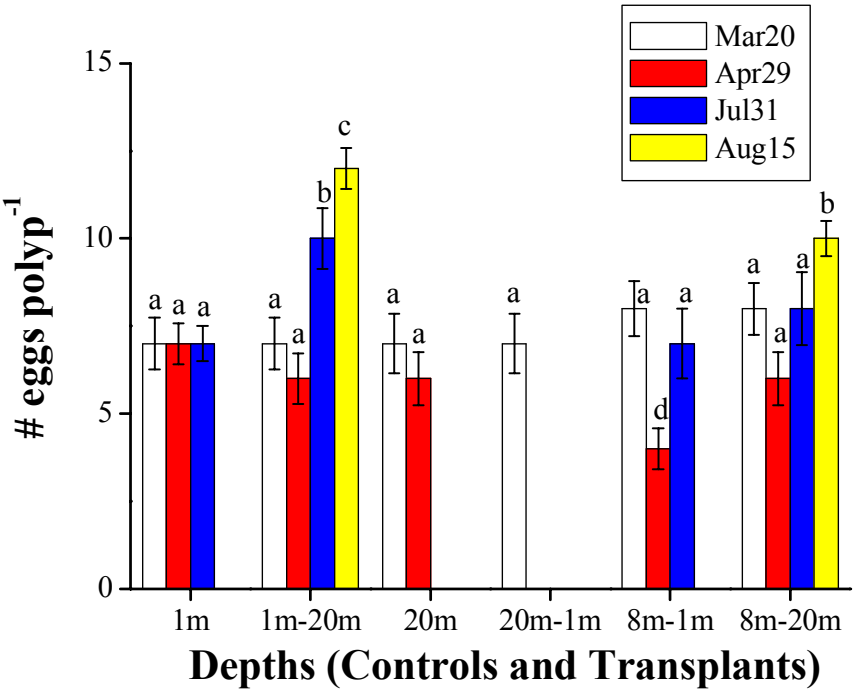


Figure 2.2. Average number of eggs per polyp of *A. cervicornis* counted using histological slides of control and transplanted colonies. n = 10 (controls and transplants). Vertical bars denote 1SD. By September 10, 2003 neither control nor transplants showed any gametes within their mesenteries. Different letters represent significantly different data ($\alpha = 0.05$).

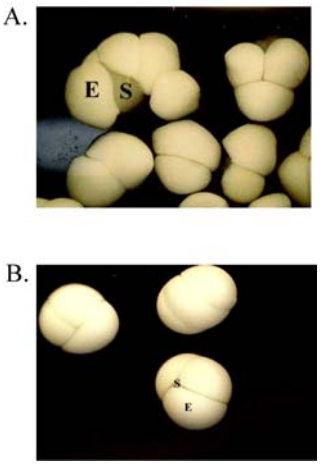


Figure 2.3. Egg bundles release by *A. cervicornis* colonies A) transplanted from El Palo Reef and B) transplanted from San Cristóbal Reef to the Old Buoys site at the shelf edge in La Parguera. S denotes a sperm package within the bundle and E marks one of the eggs for reference purposes.

None of the colonies exposed to the maximum increase in UVR (i.e., those colonies transplanted from the Old Buoy at 20m to San Cristóbal Reef at 1m) released any egg/sperm bundles. In fact, none of these colonies contained any visible eggs one month after being transplanted on March 20, 2003 and only empty holes were found in the mesenteries (Figure 2.4). Only one out of ten colonies transplanted from 20m to 1m contained a few spermaries in one mesentery by the end of July (Table 2.1). Contrarily, those colonies transplanted from shallower (1m and 8m) depths to the Old Buoy site (20m) showed a significant increase in the number of eggs per polyp (see Figure 2.3 above; Fully Nested ANOVA, $p<0.005$). Considering that these transplanted colonies from 20m to 1m depth contained visible stage II oocytes during the first sampling on March 20, 2003 just before being transplanted to 1m, the results suggest a possible re-absorption of the gametes by the parental colony tissues. This behavior has been seen in the past in bleaching-stressed colonies of Caribbean (Szmant and Gassman, 1990) and Pacific (Rinkevich and Loya, 1979) coral species. Nonetheless, the posiblility of an early expulsion of eggs either due the manipulation, increases in PAR or UVR, or any other aspect is not discarded.

Figure 2.5 shows the data for haphazardly chosen *A. cervicornis* and *P. furcata* colonies at San Cristóbal Reef (1m depth). Spawning between July and August 2003 in *A. cervicornis* colonies normally living at 1m depth at San Cristóbal Reef is reflected in the dramatic reduction in number of eggs per polyp found during the sampling on August 15. The differences between January and December suggest either a change between sequential reproductive cycles or an artifact due to the small number of colonies sampled.

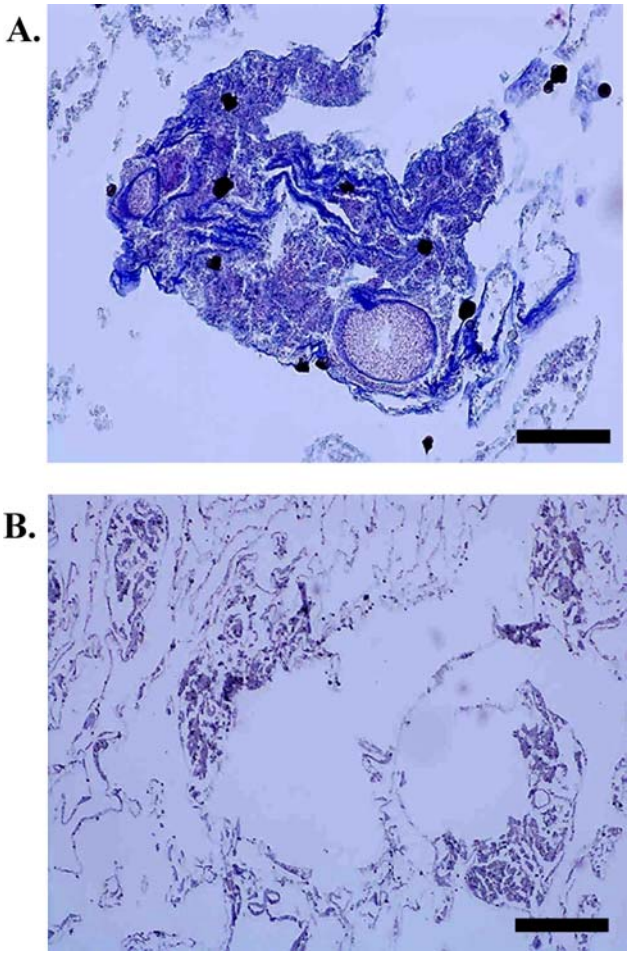


Figure 2.4. Histological sections from tissues of *A. cervicornis* colonies from the Old Buoy site (20m) show stage II oocytes a) before the transplantation and b) one month after beign transplanted to San Cristóbal Reef (1m) showing the empty spaces within the mesenteries. Scale bar = 300µm.

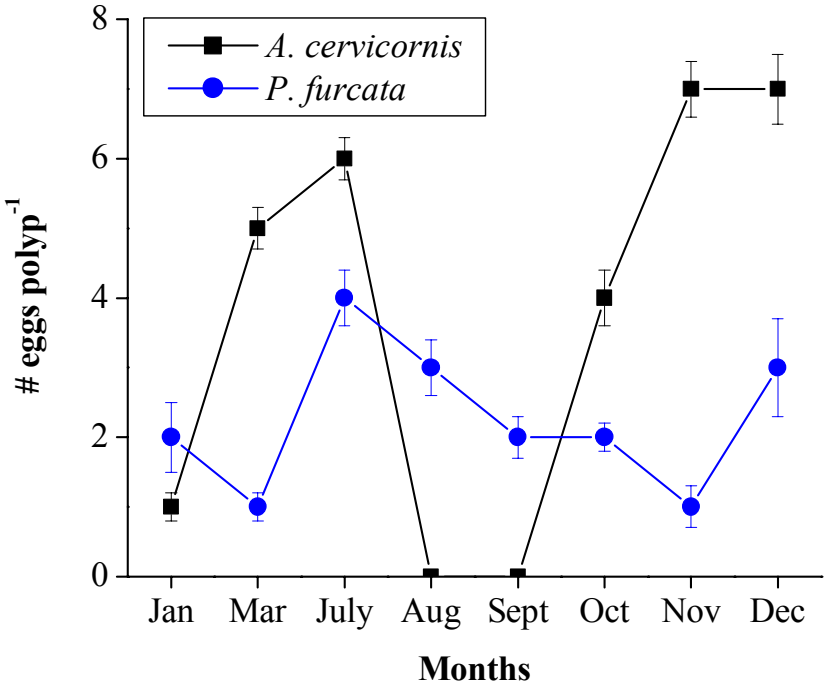


Figure 2.5. Average number of eggs per polyp of haphazardly chosen *A. cervicornis* and *P. furcata* colonies collected at San Cristóbal Reef (1m depth) during 2003. n = 4 per species per month. Vertical lines denote ± 1SD.

All *Porites furcata* colonies exposed to reduced levels of UVR in the aquariums under the Hyzod® acrylic panel contained eggs and sperm packages during all the sampling dates; no eggs were observed in those exposed to enhanced UVR during the last sampling date. Only the percentage of colonies with spermaries was reduced through out the experiment (Table 2.2) showing a dose-related behavior. However, all the colonies exposed to enhanced UVR did not contain any egs by the end of the UV-S Experiment on July 26, 2003 (Figure 2.6). Figure 2.7 shows an example of *P. furcata* tissues with gametes. The percent of colonies of *P. furcata* containing larvae varied through the experiment. While it should not be discarded, no brooding was observed in either the

transplants or the treated colonies during the experiment. *P. furcata* has been shown to release larvae several times within a single year (Soong, 1991).

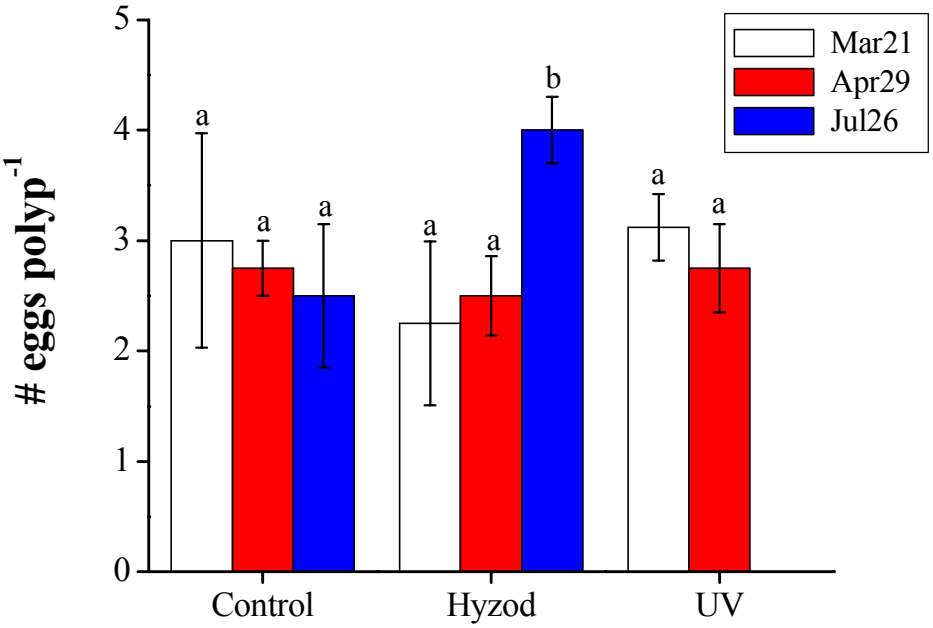


Figure 2.6 Average number of eggs per polyp of *P. furcata* counted using histological slides of control and treated colonies. n = 9 (control and treatments). Vertical bars denote 1SD. Different letters represent significantly different data ($\alpha = 0.05$).

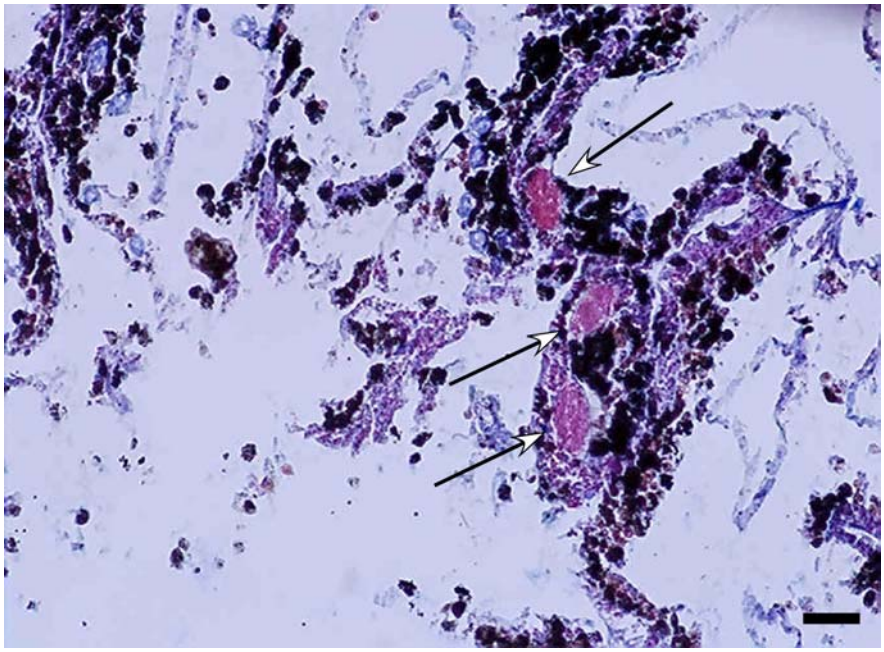

Figure 2.7. Sample of a *P. furcata* histological section showing several eggs through the polyp. Eggs are indicated by arrows. Scale bar = 150µm.

Table 2.1. Effects of changes in the UVR on the fecundity of *A. cervicornis* after being transplanted to different depths and exposed for 115 days (n=10/treatment). Roman numbers in parenthesis represent the gonad stage present at any particular sampling date. Asterisk (*) denotes only one colony (out of ten) with visible spermaries at this sampling date. Depth in meters is shown at the three different sites. Only one sampling is shown at El Palo Reef since all the colonies from this site were either transplanted to San Cristóbal Reef or to the Old Buoy site (shelf edge). Sampling on March 20, 2003 indicates the conditions of transplanted and control colonies the day before transplantation.

| Treatment/sampling date | % colonies with eggs | # eggs/mesentery | # mesenteries with eggs/polyp | % colonies with spermaries | # spermaries/ mesentery | # mesenteries with sperm/polyp |
|---|---|---|---|---|---|---|
| San Cristóbal (1m) | | | | | | |
| Mar-20-03 | 38.9 ± 13.1 (II, III) | II: 1-3; III: 1 | 1-4 | -- | -- | -- |
| Apr-29-03 | 36.7 ± 11.5 (III, IV) | III: 1-3; IV: 1-2 | 1-2 | -- | -- | -- |
| Jul-31-03 | 35.0 ± 10.9 (III, IV) | III: 1; IV: 1-3 | 1-3 | 70.0 ± 8.3 (V) | V: 7-10 | 2-3 |
| Aug-15-03 | -- | -- | -- | -- | -- | -- |
| El Palo (8m) | | | | | | |
| Mar-20-03 | 47.7 ± 12.3 (II, III) | II: 1-4; III: 1 | 1-3 | -- | -- | -- |
| Old Buoy (20m) | | | | | | |
| Mar-20-03 | 19.7 ± 3.9 (II) | II: 1-5 | 1-2 | -- | -- | -- |
| Apr-29-03 | 20.0 ± 5.3 (III) | III: 3 | 1-2 | -- | -- | -- |
| Jul-31-03 | -- | -- | -- | -- | -- | -- |
| Aug-15-03 | -- | -- | -- | -- | -- | -- |
| EP-SC (8m – 1m) | | | | | | |
| Apr-29-03 | 23.6 ± 10.4 (II, III, IV) | II: 1; III: 1-3; IV: 1 | 1 | -- | -- | -- |
| Jul-31-03 | 65.0 ± 15.6 (III, IV) | III: 1-4; IV: 1-3 | 1-2 | 67.0 ± 5.2 (V) | V: 8-17 | 1-4 |
| Aug-15-03 | -- | -- | -- | -- | -- | -- |
| OB-SC (20m – 1m) | | | | | | |
| Apr-29-03 | -- | -- | -- | -- | | |
| Jul-31-03 | -- | -- | -- | 10.0 * (III) | III: 4* | 3* |
| Aug-15-03 | -- | -- | -- | -- | -- | -- |
| SC-OB (1m – 20m) | | | | | | |
| Apr-29-03 | 33.0 ± 4.9 (IV) | IV: 1-3 | 1-2 | -- | -- | -- |
| Jul-31-03 | 60.0 ± 13.2 (IV) | IV: 1-7 | 1-3 | 50.0 ± 8.7 (V) | V: 5-24 | 1-4 |
| Aug-15-03 | 73.3 ± 10.4 (IV) | IV: 1-6 | 1-2 | 65.0 ± 7.5 (V) | V: 8-20 | 2-4 |

Table 2.1 (cont.)

| EP-OB (8m – 20m) | | | | | | |
|---|---|---|---|---|---|---|
| Apr-29-03 | 48.0 ± 7.9 (IV) | IV: 1-3 | 1-3 | -- | -- | -- |
| Jul-31-03 | 53.3 ± 5.8 (IV) | IV: 1-4 | 1-4 | 42.5 ± 1.8 (V) | V: 9-10 | 2-3 |
| Aug-15-03 | 33.3 ± 12.6 (IV) | IV: 1-6 | 1-4 | 45.0 ± 11.1 (V) | V: 10 | 2 |

Table 2.2. Effects of changes in the UVR on the fecundity of *P. furcata* maintained at the outdoor aquariums for 128 days (n=9/treatment).

| Treatment/sampling date | % colonies with eggs | % colonies with spermaries | % colonies with larvae |
|---|---|---|---|
| Control | | | |
| 4/7/03 | 88.9 | 77.8 | 44.4 |
| 4/29/03 | 100 | 100 | 0 |
| 7/26/03 | 100 | 100 | 66.7 |
| Hyzod | | | |
| 4/7/03 | 100 | 100 | 33.3 |
| 4/29/03 | 100 | 100 | 0 |
| 7/26/03 | 100 | 100 | 77.8 |
| UV | | | |
| 4/7/03 | 100 | 100 | 33.3 |
| 4/29/03 | 100 | 50 | 0 |
| 7/26/03 | 0 | 33.3 | 0 |

## DISCUSSION

The present work shows the effects of enhanced UVR (either naturally as in the case of *A. cervicornis* or artificially as in the case of *P. furcata*) on the fecundity of ramose Caribbean coral species. During the UV-S Experiment on *A. cervicornis*, the colonies transplanted from 1m and 8m to 20m delayed their reproductive cycles and spawned approximately two-three weeks after the control colonies of the shallower site and approximately one month after the control colonies at their transplanted site (20m). Both represent a delay to the next lunar cycle. Contrarily, the reproductive structures of those colonies transplanted from 20m to 1m were not visible one month after the transplant or during the rest of the experiment. The colonies transplanted from 8m to 1m did not show any reduction in fecundity. These colonies spawned at the same time of the control ones at shallow depth as evidenced on August 15, but since no control colonies

were sampled at El Palo Reef after the first sampling date, no assumption can be made as to whether the transplanted colonies from El Palo Reef (8m) to San Cristóbal Reef (1m) changed their spawning behavior or spawned at their "normal" time. The control colonies of San Cristóbal and those transplanted from El Palo Reef to San Cristóbal Reef both contained stage IV oocytes on July 31$^{st}$, but no colonies contained any gametes on August 15 indicating a possible spawning event between July 31$^{st}$ and August 15$^{th}$ 2003; this is, between the new moon of July 29$^{th}$ and the full moon of August 12$^{th}$. Hence, the variability in spawning times of this species within its depth range should be considered when predicting *A. cervicornis* spawning events and in future reproductive studies.

The delay in spawning times in transplanted colonies from shallow and intermediate depths to deeper areas compared to the controls at 1m and 20m depth reflects either an adaptation strategy to ensure reproductive success in a different environment, a consequence of stress caused by the transplanting manipulation, or both. The former can be discarded considering that the control colonies at 20m spawned approximately one month before the transplanted colonies. The latter reflects a change in their diel cycle as these colonies were suddenly submitted to lower irradiances, and hence, less daylight hours. The delay in spawning times in the colonies transplanted from shallow to deeper waters could be the result of the phenotypic plasticity characteristic of reef corals (Soong, 1991). Further studies must be performed to confirm these hypotheses.

Spawning in shallow living *A. cervicornis* colonies after the new moon relative to those colonies of the same species living at deeper areas suggests a relationship to the spring tides produced during the new moon. Yet, it would be expected that these colonies

might have spawned either after the new or full moon since both phases are related to these high tides (Brown et al., 1989). Spawning during spring tides can be more advantageous to those corals living at shallow areas since water column height above the colonies increases and, hence, the penetration of UVR decreases. The increase in the water column also aids the success in recruitment and planula larvae settlement, as these would be exposed to reduced UVR levels. It has been demonstrated larvae produced at different depths respond differently relative to their sensitivity to UVR (Gleason and Wellington, 1995). UVR reduction related to spring tides might have little or no effect, on the other hand, on naturally living deeper colonies since the amount of UVR reaching these depths (20m) is minimal (see Figure 11 on the Experimental setup and characterization of the underwater light field section).

The delay in spawning times in colonies transplanted to deeper areas compared to those living at shallow areas suggests an effect of the amount of PAR reaching the colonies more than an effect of the reduced UVR. A reduction of PAR means less visible light available for the photosynthetic processes of the zooxanthellae and, hence, less overall energy available to the scleractinian host. Kojis and Quinn (1984) and Jokiel (1985) studied the effect of reduced PAR on coral fecundity. Both studies suggested that lower irradiances are one of the most important environmental factors affecting the reproduction of *Pocillopora damicornis* in Hawaii.

Bleaching either caused by increases in temperature or light intensities, including UVR, can affect coral reproduction. Depending on the intensity of the bleaching event, the reproductive output of cnidarians can be reflected for at least two spawning seasons.

Michalek-Wagner and Willis (2001a) found significantly reduced levels of fecundity and eggs sizes in the soft coral *Lobophytum compactum* 20 months after a bleaching episode.

The severe bleaching that occurred to the colonies transplanted from 20m to 1m just after one day of the transplant might have been one of the causes for the disappearance of the coral gonads in *A. cervicornis*. Davies (1991) estimated that corals could survive periods of decreased photosynthetic productivity between 1-4 months through catabolism of lipid resources, such as gametes. Similarly, Michalek-Warner and Willis (2001b) reported a two-fold reduction in lipids, proteins and carotenoids in eggs of the soft coral *Lobophytum compactum* after a bleaching event. Although lipids were not measured during the present study, the 100% reduction in the fecundity of [bleached] colonies transplanted from 20m to 1m depth strongly suggests that survival occurred at a significant expense of those resources that would have normally been allocated for continuing sexual reproduction. This further indicates that the reproductive parameter measured (i.e. fecundity) can be used as an indicator of sub-lethal stress in reef corals.

Experiments performed with other *Acropora* species in Japan further confirm the hypothesis of re-absorption of lipids reserves. Colonies of *Acropora nasuta* from Okinawa decreased their fertilization rates by 52% after the mass bleaching event of 1998 (Omori, et al., 2001). The authors did not explain whether the lowered reproduction of the corals was caused by the temporary loss of zooxanthellae during the bleaching that occurred 9 months earlier. It is likely that a drop in fertilization rates was followed by a reduction in coral recruitment of *A. nasuta*. Szmant and Gassman (1990) studied colonies of the Caribbean reef-builder *Montastraea annularis* after the 1987 Caribbean wide bleaching event. They found that bleached colonies were not able to complete

gametogenesis during their reproductive season of that year following the bleaching, while normally colored corals completed their cycle. The authors proposed that bleached corals might have been able to survive even with the reduced concentrations of zooxanthellae by consuming their own structural materials reducing the resources necessary for reproduction.

The present findings, especially in *A. cervicornis*, where no gametes were seen after the bleaching resulting from the transplants to shallower areas, provide additional evidence for the re-absorption of reproductive materials proposed by Szmant and Gassman (1990) for Atlantic corals and Ward (1995) for Pacific corals to survive the bleaching stress. Furthermore, it suggests that the algal translocated products might be used by the coral not only for growth but possibly as an energy source for gametogenesis, especially considering that the algal symbiont translocates up to 95% of its photsynthates to the coral host, thereby providing up to 143% of its daily energetic costs (Muscatine et al., 1981; Davies, 1991; Michalek-Warner and Willis, 2001a). Nonetheless, other reactions to the stress event such as the early expulsion of the gametes cannot be discarded.

Most of the experiments relating UVR and invertebrate reproduction have been performed in the past by exposing either the larvae or developing embryos to changes in UVR instead of exposing the parent colonies which will ultimately release, or not, the gonads to the environment. Gleason and Wellington (1995) attributed the differences in survivorship in the larvae of *Agaricia agaricites* exposed to higher levels of UVR and PAR during a transplant experiment to differences in the concentration of MAA's found in the larvae. At 3m depth, larvae exposed to PAR + UVA + UVB showed a 74%

decrease in survival rate and significant reductions in the chlorophyll concentrations than larvae exposed to either PAR + UVA or PAR alone. Kuffner (2001b) reported a negative effect on larvae settlement in *Pocillopora damicornis*; however, the author did not find any increase in mortality after exposure to UV-transparent conditions.

Lesser and Barry (2003) observed significant decreases in survivorship, delays in development, and abnormal embryos and larvae of echinoderms associated with exposure to UVR. Also, the percent survival of sand dollar embryos rapidly decline under UVA and UV transparent compared to UV occluded treatments. Embryos exposed to UVR exhibit significant mortality and the survivors are likely more resistant to the lethal effects of UVR. They also found up to 20% of abnormal embryos or larvae after exposure to UVR apparently due to DNA damage in the developmental stages (Lesser and Barry 2003). Adams and Shick (2001) also reported as high as 50% increase in the presence of abnormal larvae and embryos as a consequence of UV-B in the green sea urchin *Strongylocentrotus droebachiensis*. Both studies agree that later stages of development may be more tolerant with less DNA damage than early stages. This might also account for the significant reduction in gametes seen in both species during the present study when exposed to increased levels of UVR.

It is acknowledged here that factors affecting the survivorship of hermatypic corals planulae larvae while in the plankton will ultimately have an impact on reef growth and sustainability (Gleason and Wellington, 1995). Nevertheless, the present study shows that colonies of the same broadcasting species living at different depths may release their gametes (e.g., spawn) during different times. This behavior might either be related to differences in the water column height and consequently on the UVR levels reaching the

coral colonies, or to the differences in the amount of PAR reaching the colonies, or both. More studies are needed to investigate which of these factors is a greater contributor to the reproductive success of reef corals. Furthermore, it is shown that a sudden increase in UVR can fully stop coral sexual reproduction by forcing the coral animals to re-absorb the materials dedicated to reproduction to fulfill their metabolic demands when living under such stressful conditions (i.e. by increasing the amount of photoprotective compounds).

In summary, coral species, either brooding or broadcasting, may respond differently to enhanced UVR. Broadcasting colonies exposed to a sudden increase in UVR may reduce their gamete contains completely either due to a re-absorption of the gametes to be used as an alternative source of energy to be allocated for the production of UV-absorbing compounds, or as an expulsion of the gametes. Brooding colonies, however, are apparently more resistant to changes in their respective reproductive structures as a result of increased UVR and effects may not readily be seen in short-term studies. Further longer-term studies must be performed to fully assess the response of the reproduction and fecundity of scleractinian corals to different environmental parameters including changes in the light regime.

# CHAPTER III. EFFECTS OF CHANGES IN UV RADIATION ON ZOOXANTHELLAE DENSITY AND PIGMENTS PRODUCTION OF *ACROPORA CERVICORNIS* AND *PORITES FURCATA*

## ABSTRACT

Eighteen colonies of *Acropora cervicornis* and *Porites furcata* were exposed to decreased levels of UVR and PAR with Hyzod® acrylic panels and Saran® meshes at 1.5m depth in La Parguera. The identification and quantification of all pigments was performed with HPLC analysis. The concentration of UV-absorbing compounds (MAA's) decreased significantly in both species compared to controls expose to normal UVR and PAR levels, while photosynthetic pigments concentrations showed an inverse relationship with PAR levels reaching the colonies. Those colonies exposed to decreased UVR and PAR levels contained significantly higher concentrations of chlorophyll *a*, chlorophyll $c_2$, and peridinin. Zooxanthellae density among treatments was not significantly different, but an increasing trend was observed with decreasing UVR and PAR levels. Each species was also treated with increased UVR levels either naturally or artificially by either transplanting ten *A. cervicornis* colonies from deep (20m) and ten from intermediate (8m) to shallow areas (1m), or transplanting nine colonies of *P. furcata* to an open system outdoor aquaria with enhanced daily UVR doses provided by Q-Panel UV-313nm fluorescent lamps. Colonies transplanted to shallow areas showed an immediate bleaching event on the sun-facing sides of their respective branches. The bottom-facing side of the branches was not affected, and re-coloration of the sun-facing

sides after three months suggests an active transport of zooxanthellae cells from unaffected to affected areas aided by a significant increase in the mitotic index of the remaining surviving cells at the affected sides. MAA's concentrations significantly increased in colonies of both species exposed to enhanced UVR levels including in the severely bleached colonies. While colonies contained similar MAA's, the individual response of these compounds varied between species with palythine ($\lambda_{max}$=320nm) predominating in *A. cervicornis* and mycosporine-glycine ($\lambda_{max}$=310nm) in *P. furcata*. The differences in MAA's and accessory photosynthetic pigments in both species suggest the presence of different zooxanthellae clades in both species. The results show that former experiments that extrapolated possible effects of increased UVR by submitting the colonies to UVR-depleted treatments might underestimate the possible real coral responses to increases in UVR daily doses.

## INTRODUCTION

The visible component of sunlight (400-700nm, PAR) is not greatly attenuated by the atmosphere (Calkins, 1982). Contrarily, the UVR portions, especially the shorter and more biologically injurious UV-B wavelengths (280-320nm) are strongly absorbed by the stratospheric ozone layer (Calkins and Thordardottir, 1980). Hence, the levels reaching the earth's surface are significantly reduced compared to the longer wavelengths (UV-A, 320-400nm). Nevertheless, they are sufficient to produce significant damage to organisms either terrestrial or marine.

Studies on the effects of UVR on reef corals, especially on the production of UV-absorbing compounds (Mycosporine-like Amino Acids or MAA's), performed during the past decades have explored how these organisms change their MAA's concentrations by decreasing the levels of UVR reaching the experimental colonies (Grottoli-Everett and Kuffner, 1995; Lewis, 1995) . In this sense, the effects of an increase in UVR due to a thinning of the earth's ozone layer are assumed to be the contrary to the obtained results. Very few investigators have presented experimental designs where the amount of UVR is physically increased either naturally or artificially. Gleason and Wellington (1993), for example, experimentally "increased" levels of UVR reaching colonies of the brown and green morphs of *Porites astreoides* was accomplished by transplanting the colonies from deeper to shallow areas.

This study presents both types of experiments: one where the colonies are exposed to decreased UVR levels (UV-Exclusion Experiment or UV-E), and another where the colonies are exposed to enhanced UVR levels either naturally or artificially (UV-Supplementation Experiment or UV-S). Therefore, this is the first study to compare both types of results and, at the same time, explores the feasibility of the formerly explained setups. This chapter presents the effects of changes in ultraviolet radiation on the photosynthetic and photoprotective pigments concentration with emphasis on the MAA's. Also, probable bleaching events that might have occurred during the study are explored in terms of both zooxanthellae densities and photosynthetic pigments concentrations decreases.

# METHODS

Identical protocols were used during the UV-E and UV-S Experiments for the extraction and quantification of photosynthetic pigments, MAA's, and zooxanthellae density analysis. Therefore, the following methodological description is not separated into the two different experiments. Nonetheless, the results are presented in terms of both experiments.

## Sampling and extraction

One branch from each colony (both species) was removed during each of the three sampling dates (beginning, one month after, and end of the experiment). All samples were transported in sterilized Whirl-Pak plastic bags. Each branch was broken into three pieces. The proximal piece (closer to the base of the branch) was used for histological analysis, the middle was used for zooxanthellae density and the distal piece was used for the pigment analysis. The tip of each branch was discarded due to either lack of zooxanthellae or pigments (Oliver, 1984). Also, this piece usually does not contain any reproductive tissue and is mainly used by branching corals for linear growth (Gladfelter, 1982; Oliver, 1984).

Length and width of each fragment was measured with a Vernier caliper to the nearest 0.1mm prior to the pigment extraction and used to obtain an areal estimation of the coral tissue. This measure was later used for normalization of photosynthetic pigments concentration. Individual samples were then placed into 40ml crystal vials.

Samples were extracted following the procedures of Chalker and Dunlap (1981) as implemented locally by Muszynski et al. (1998) and by Corredor et al. (2000) using 20ml of an HPLC grade menthanol:tetrahydrofuran (80:20, v/v) solution. Both temperature and methanol concentration can affect the extraction efficiency (Tartarotti and Sommaruga, 2002); hence, the best methanol formula was used and all extractions were performed at 4ºC (Chalker and Dunlap, 1981).

The first extraction was performed for 24hrs at 4ºC followed by a second 20min extraction to remove any pigments left. Both extractions were performed in the dark, by covering the sampling tubes with aluminum foil, to avoid any pigment degradation (Jeffrey, et al., 1974). Corals were extracted the same day of field sampling to avoid any diatoxanthin-diadinoxanthin (DT-DD) interconversion prior to analysis (Bidigare, 1991).

A spectrophotometric analysis was performed using a Hewlett-Packard HP8452 spectrophotometer for each sample. Photosynthetic pigments were separated from MAA's using Sep-Pak $C_{18}$ 900mg cartridges. In this procedure, photosynthetic pigments are retained in the cartridge. These were then removed from the cartridge by injecting 5ml of the extraction solvent. The different pigments were identified using reversed-phase High Performance Liquid Chromatography (HPLC). Both photosynthetic and MAA's were kept in 4ml HPLC vials until HPLC analysis was performed. All samples were stored at -70ºC before HPLC analysis.

## High Performance Liquid Chromatography (HPLC)

### Mycosporine-like Amino Acids (MAA's)

Mycosporine-like amino acids were separated following published extraction protocols described by Gleason (1993) and later by Muszynski et al. (1998). Briefly, MAA's were separated by injecting 30-80 µl in a Phenosphere® 250 x 4.6mm $C_8$ column connected to a Shimadzu LC-10AT Liquid Chromatograph coupled to a Shimadzu SPD-M10AV diode-array detector using an isocratic solution consisting of 55%MeOH, 0.1%Acetic Acid and 44.9%DIW ran at 0.8ml/minute for 20 minutes. Samples were injected with a Waters 712 WISP Autosampler. Due to the high concentration, especially of samples from San Cristóbal Reef and from the outdoor aquariums, some samples were diluted to a 1:10 solution using the extracting solvent before the injection into the HPLC. Samples from the two species were sent to Dr. Daniel Gleason (Georgia Southern University) for MAA's identification. Samples were co-cromatographed with standards obtained from *Porites astreoides* from St. Croix, *Lissoclinum patella* and *Porphyra* sp. from Australia, and *Acanthopleura elegantissima* from Pacific Grove, California (Daniel Gleason, personal communication). MAA's concentrations were normalized to soluble protein from an aliquot of the extracted sample. Protein concentrations were determined following the procedure of Bradford (1976; see below).

### Photosynthetic Pigments

Photosynthetic pigments were separated from the UV-absorbing compounds by injecting the extracts through a Sep-Pak $C_{18}$ cartridge following the procedures of Shick et al., (1995). The photosynthetic fraction was then removed from the filters by injecting

5 ml of the extraction solvent. Absorbance measurements (400-700 nm) were obtained with a Hewlett-Packard 8452A diode-array spectrophotometer. Reversed-phase HPLC analysis was performed in a Shimadzu LC-10AT Liquid Chromatograph coupled to a Shimadzu SPD-M10AV diode-array detector to separate the different photosynthetic pigments. Samples were injected with a Waters 712 WISP Autosampler. Reversed-phase conditions are preferred to normal phase because the polar stationary phases of the latter promote pigment degradation. Pigments were separated using a modification of the procedure described by Wright et al. (1991). The gradient system consisted of 80:20 methanol:ammonium acetate (pH 7.2, v/v), 90:10 acetonitrile:water, and 100% ethyl acetate with a Symmetry ® $C_{18}$, 25 cm x 3.9 mm-inner diameter, 5 µm particle size column at a constant flow rate of 1.0 ml $min^{-1}$. Eluting peaks were detected using the absorbance spectra at 436 nm for carotenoids and chlorophylls (Bidigare, 1991). Peaks were integrated, and quantification of individual pigments was accomplished using peak areas and calibration factors determined with authentic standards of chl *a* and lycopene (Sigma Co.). Individual pigments were identified using published signatures and their respective peak maxima (Wright et al., 1991; Jeffrey et al., 1997). Photosynthetic pigments and zooxanthellae concentrations were normalized to coral tissue area determined by the aluminum foil technique (Marsh, 1970). Photosynthetic pigments were also normalized to zooxanthellae densities. Concentrations are expressed in µg $cm^{-2}$ or pg zooxanthellae $cell^{-1}$.

**Zooxanthellae analysis**

The zooxanthellae density can be used as a consistent predictor of bleaching (Stimson et al., 2002). The middle piece of the sampled branch was used for this purpose. The tissue was fixed using a 10% Formalin in seawater solution for 24hrs and rinsed in DIW for another 24hrs. Fixation was performed at similar hours during the sampling dates to avoid any influence from the diel patterns of cell division (Jones, 1997). Then, decalcified using a 10%HCL 0.7%EDTA solution. The tissue cylinder was grounded with a mortar and pestle and homogenized at 7,000rpm with a tissue homogenizer (Biospec Products, Inc.). The slurry was decanted into a 50ml centrifuge tube with 5ml of DIW, centrifuged at 5,000rpm for 15 minutes, and the supernatant discarded. The remaining pellet containing the zooxanthellae was resuspended in 2ml of DIW until analysis. Counts were performed in triplicate in a Reichert haemacytometer and averaged. The same protocol was used during both experiments. The number of dividing cells was also noted for later estimation of the Mitotic Index (MI) (Wilkerson et al., 1983, 1988). Assessing changes in mitotic index has been proposed as a potential means of assessing stress in corals (Brown and Howard, 1985; Brown, 1988).

**Protein assay analysis**

Protein analysis was performed using a Bio-Rad soluble protein determination kit. The procedure is similar to the one described by Bradford (1976). Protein standards were prepared with lyophilized Bovine Gamma Globulin. The solid portion of each sample was transferred to 15ml centrifuge tubes with 3ml NaOH 1N and heated for 30 minutes at 90$^{o}$C to solubilize the proteins. Samples were allowed to cool down at room temperature

for approximately 1hr and then neutralized with 3ml HCL 1N. 100ul aliquots of each solution were assayed with 500ul of the alkaline copper tartrate solution (Reagent A), and 4ml of a dilute Folin Reagent (Reagent B) and shaken in a Vortex mixer. After 15min the absorption was recorded at 750nm in a Shimadzu UV-Visible UV-1601 spectrophotometer.

## RESULTS

### UV-E Experiment

#### Mycosporine-like Amino Acids (MAA's)

A significant reduction was found in the total concentration of MAA's in both species exposed to reduced UVR levels in the field (Figure 3.1A, *A. cervicornis*: Fully Nested ANOVA, $p<<0.0001$; Figure 3.1B, *P. furcata*: Fully Nested ANOVA, $p<0.0001$). No differences in total MAA's were found between replicates among treatments for both species [Fully Nested ANOVA, (*A. cervicornis*, $p=0.938$) (*P. furcata*, $p=0.962$)]. Total MAA's were reduced to a 16% of that of the control colonies in *A. cervicornis* in those colonies placed under the Hyzod® or Saran® treatments ($64.53 \pm 6.6$ and $65.73 \pm 13.0$ nmol mg protein$^{-1}$, respectively, compared to $400.92 \pm 35.7$ in the control colonies). Similarly, total MAA's were reduced to 12 and 19% in the colonies of *P. furcata* placed under the Hyzod® or Saran® treatments, respectively, compared to the control colonies ($17.0 \pm 6.8$ nmol mg protein$^{-1}$ in Hyzod®; $25.8 \pm 4.0$ nmol mg protein$^{-1}$ in Saran®; and $137.9 \pm 19.1$ nmol mg protein$^{-1}$ in the control colonies). The relationship between total MAA's in both species and total UVR measured at San Cristóbal Reef during the UV-E

Experiment is shown in Figure 3.2. Total MAA's of both species were significantly lower under 95-99% depleted UVR compared to controls exposed to actual UVR levels (t-test: $p<0.0001$ for *A. cervicornis* and $p<0.0001$ for *P. furcata*).

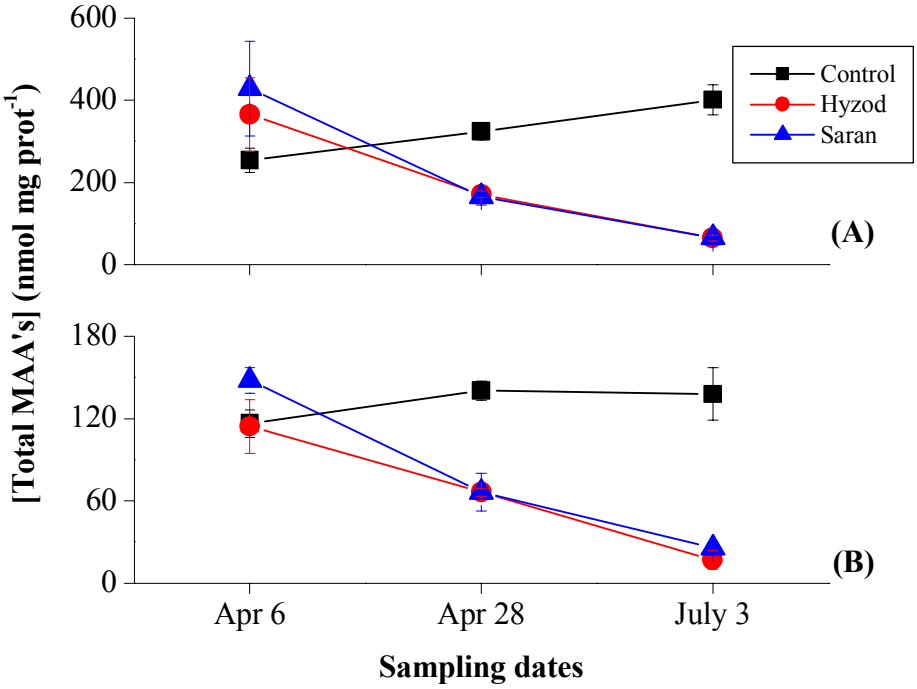


Figure 3.1. Total MAA's concentration in A) *A. cervicornis*, and B) *P. furcata* (nmol mg protein$^{-1}$) measured during the UV-E Experiment. Vertical lines denote ±1SD.

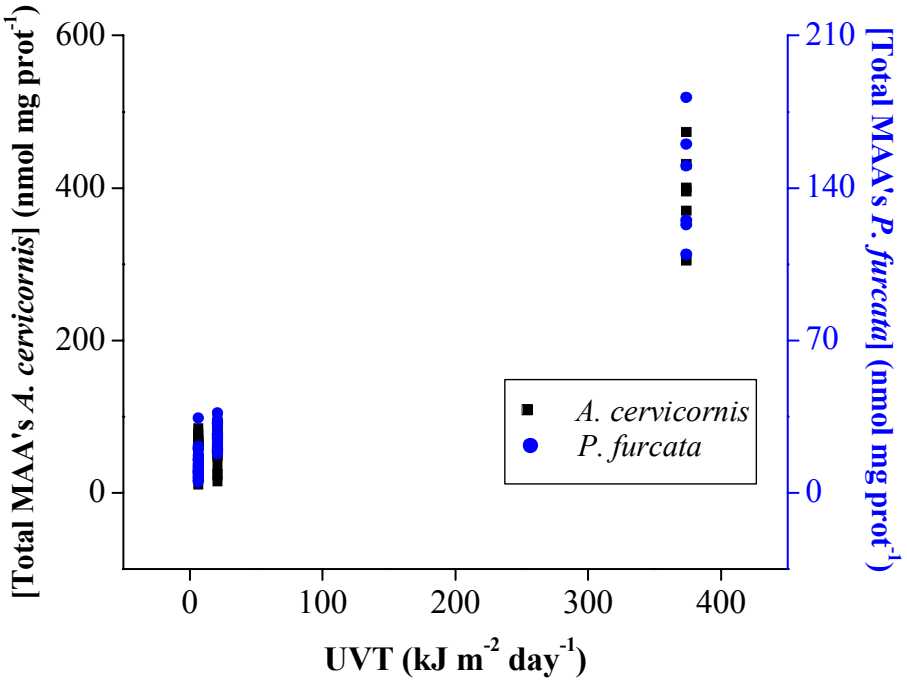


Figure 3.2. Relationship between total concentration of MAA's and UVR measured at San Cristóbal Reef during the UV-E Experiment.

The following MAA's were identified in *A. cervicornis*: mycosporine-taurine (λmax = 309nm), mycosporine-glycine (λmax = 310nm), palythine (λmax = 320nm), asterina-330 (λmax = 330nm), shinorine (λmax = 333nm), porphyra-334 (λmax = 334nm), palythene (λmax = 360nm); while *P. furcata* contained: mycosporine-taurine, mycosporine-glycine, palythine, asterina-330, shinorine and porphyra-334. These pigments were constant in both species during the UV-E and UV-S Experiments. Due to a difference in the mobile phase used by Dr. Danny Gleason (30%MeOH instead of the 55%MeOH used here), usujirene (λmax = 357nm) was identified in *A. cervicornis* using published retention times and spectrum characteristics.

Palythine was the dominant MAA found in *Acropora cervicornis* followed (in order of dominance) by asterina-330, mycosporine-glycine, palythene, shinorine, usujirene, and porphyra-334. In *Porites furcata*, mycosporine-glycine was the dominant MAA found followed (in order of dominance) by asterina-330, porphyra-334, shinorine, and palythine. A representative chromatogram showing the different MAA's found in both species is shown in Figure 3.3. A few colonies of both species contained traces of mycosporine-taurine. Stochaj, et al. (1994) first described this compound for the sea anemone *Anthopleura ellegantissima*. Individual MAA's concentrations are shown in Figures 3.4-3.5 for *Acropora cervicornis*, and Figures 3.6-3.7 for *Porites furcata*. None of the MAA's that absorb beyond 340nm (i.e., palythene and usujirene) were found in *P. furcata*.

In *Acropora cervicornis*, all MAA's showed a systematic decrease through the UV-E Experiment in colonies exposed to reduced levels of UVR. By the end of the experiment, none of the colonies in the Saran® treatment contained palythene, and only one colony contained a small amount of porphyra-334. Similarly, only one colony in the Hyzod® treatment contained a small amount of shinorine by the end of the experiment. In contrast, the control colonies showed an increase in all the MAA's concentration, associated with an increase in both solar day and UVR doses as the experiment approached summertime.

In *Porites furcata*, the effect of reducing the UVR levels reaching the colonies varied depending on the individual MAA. For example, while mycosporine-glycine and asterina-330 concentrations were reduced throughout the experiment in both treatments, porphyra-334 and palythine showed a slight increase under the Hyzod® after one month

of exposure. Yet, none of the colonies contained shinorine, asterina-330, and porphyra-334 by the end of the experiment. Only the concentrations of mycosporine-glycine and porphyra-334 were systematically increased throughout the experiment in the control colonies of this species. The concentration of the other three MAA's present in *P. furcata* (shinorine, asterina-330, and palythine) varied throughout the experiment and showed a reduction at the end.

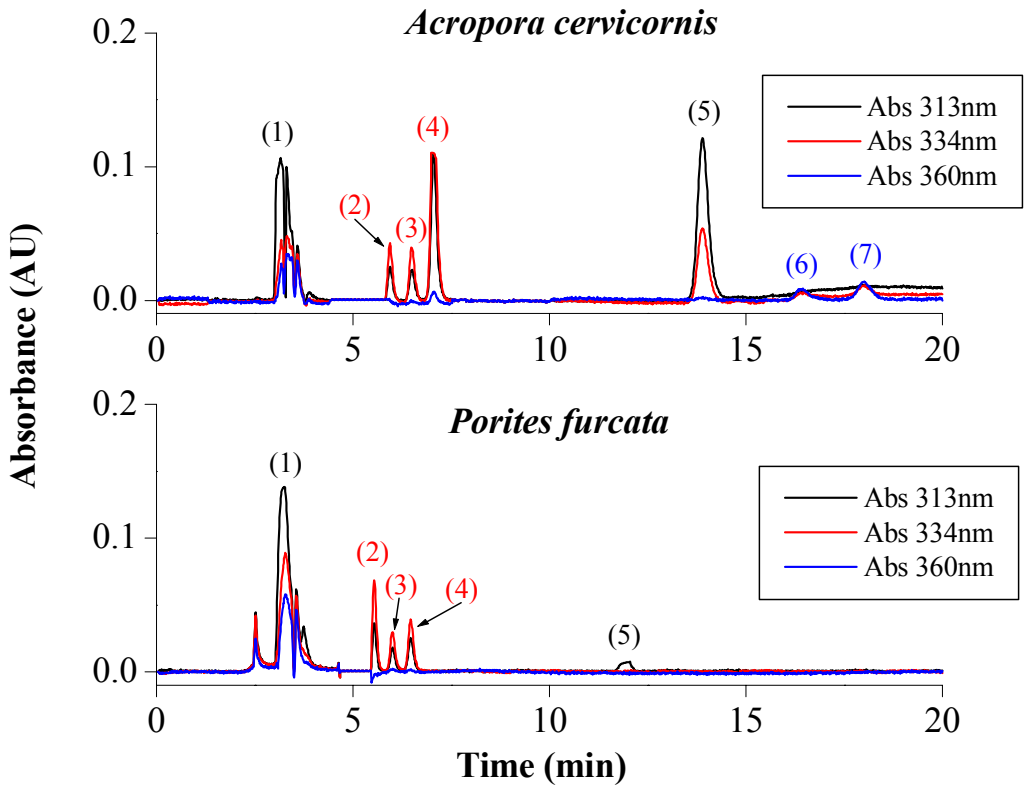


Figure 3.3. Representative chromatogram showing the different MAA's found in both species. The colors of the numbers indicate, according to the legend, at which wavelength absorbance was read for each pigment. (1) = mycosporine-glycine (λmax = 310nm); (2) = porphyra-334 (λmax = 334nm); (3) = shinorine (λmax = 333nm); (4) = asterina-330 (λmax = 330nm); (5) = palythine (λmax = 320nm); (6) = usujirene (λmax = 357nm); and (7) = palythene (λmax = 360nm).

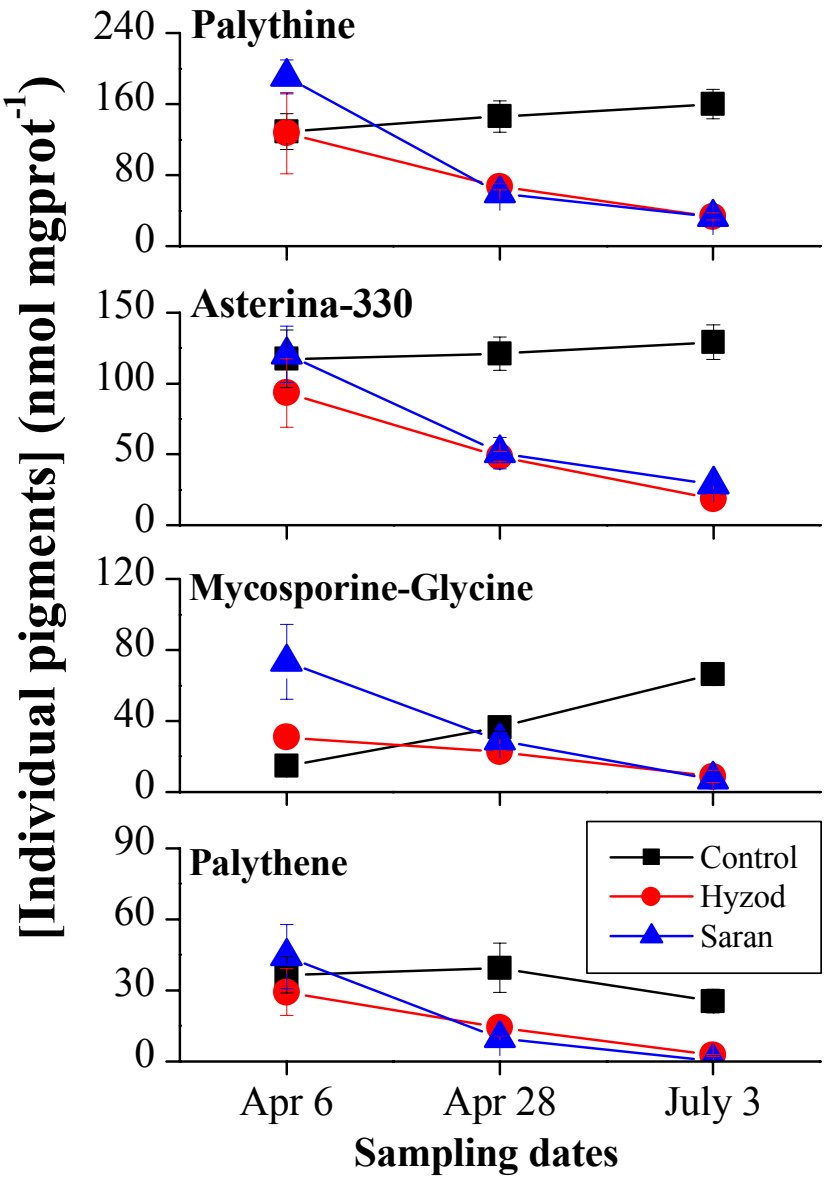


Figure 3.4. Concentration of palythine (λmax = 320nm), asterina-330 (λmax = 330nm), mycosporine-glycine (λmax = 310nm), and palythene (λmax = 360nm) in *A. cervicornis* during the UV-E Experiment. Vertical lines denote ±1SD.

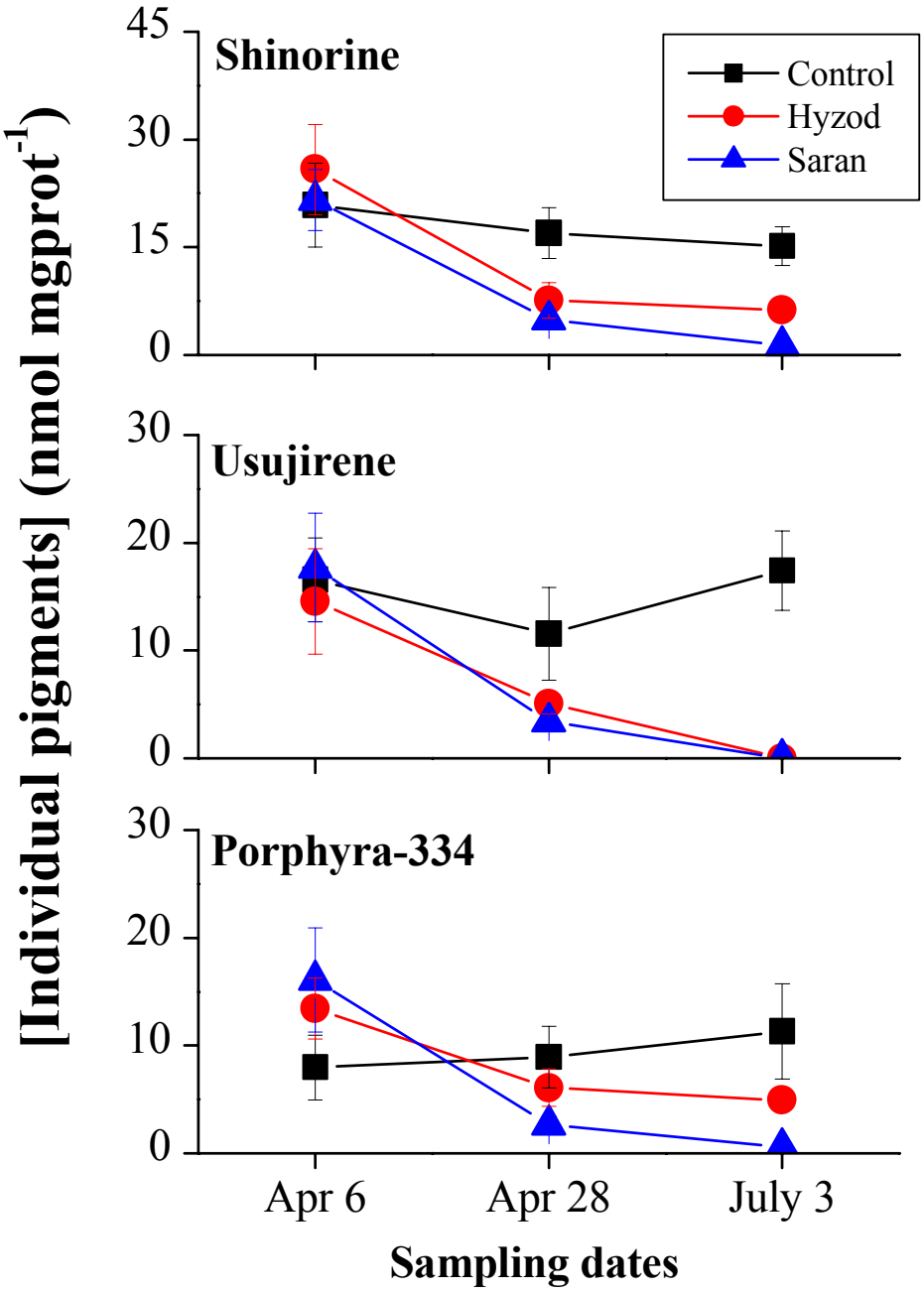


Figure 3.5. Concentration of shinorine (λmax = 333nm), usujirene (λmax = 357nm), and porphyra-334 (λmax = 334nm) in *A. cervicornis* during the UV-E Experiment. Vertical lines denote ±1SD.

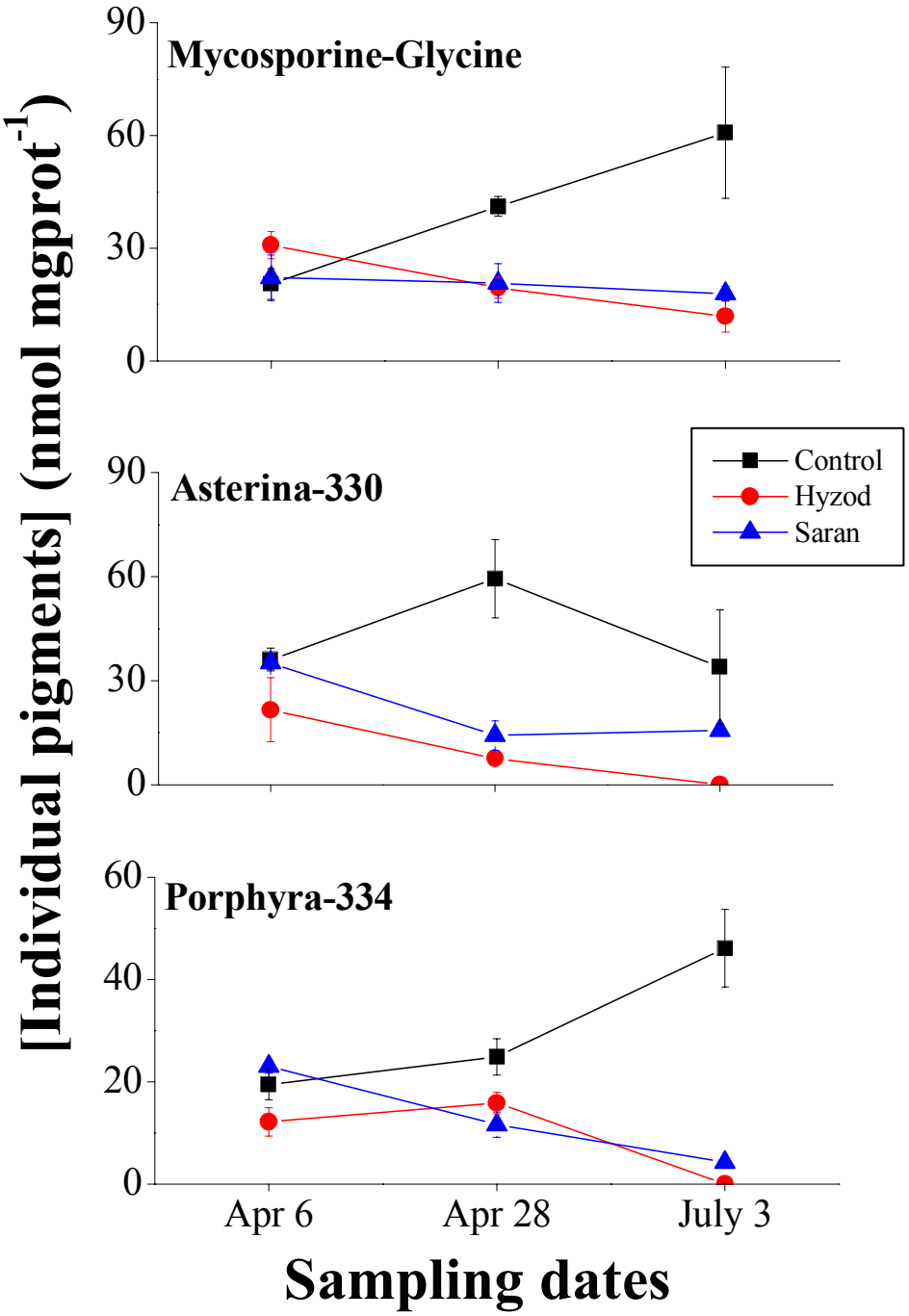


Figure 3.6. Concentration of mycosporine-glycine (λmax = 310nm), asterina-330 (λmax = 330nm), and porphyra-334 (λmax = 334nm) in *P. furcata* during the UV-E Experiment. Vertical lines denote ±1SD.

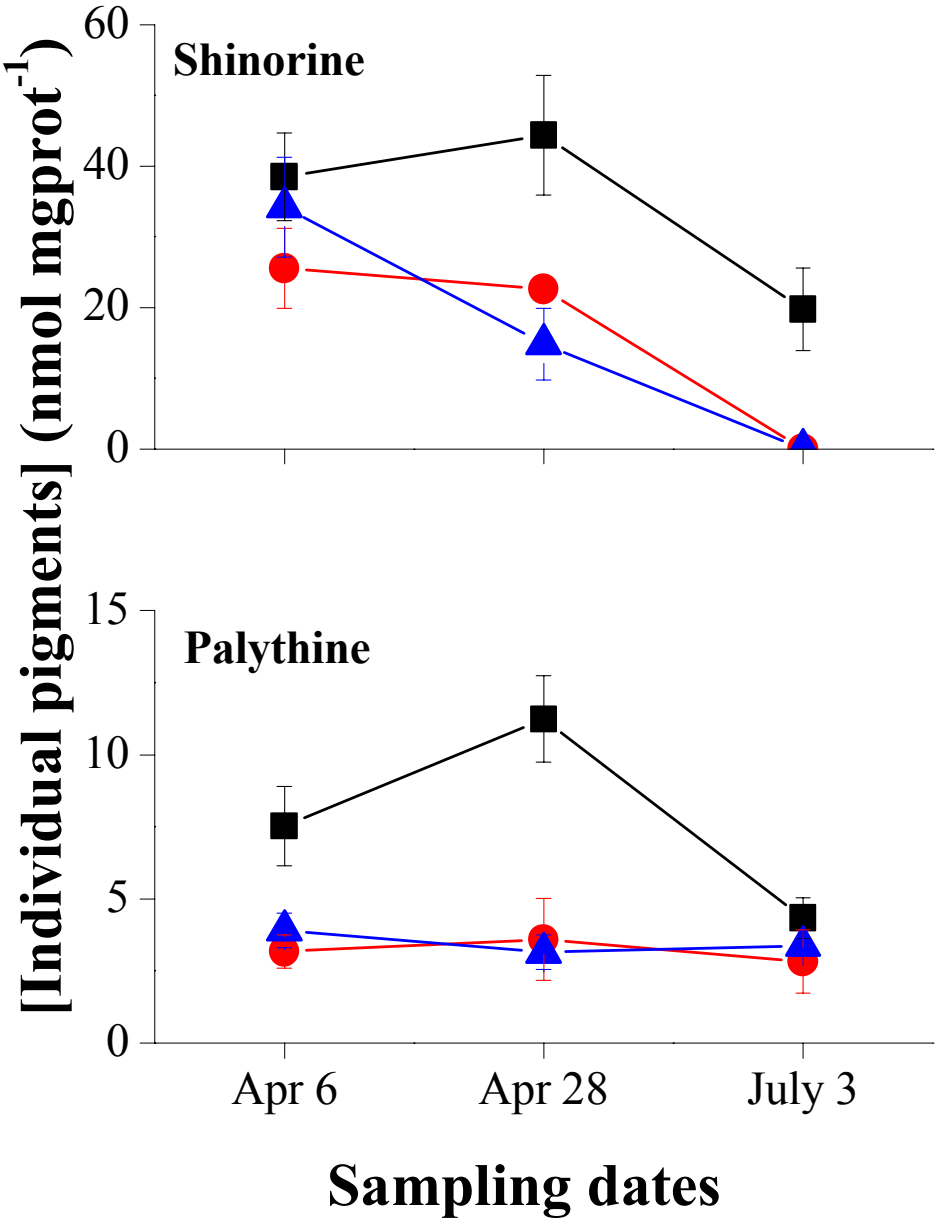


Figure 3.7. Concentration of shinorine (λmax = 333nm) and palythine (λmax = 320nm) in *P. furcata* during the UV-E Experiment. Vertical lines denote ±1SD.

**Photosynthetic pigments**

The total amount of photosynthetic pigments per host tissue area in *Acropora cervicornis* showed a significant increase in the Saran® treatment compared to the Hyzod® and control colonies (Figure 3.8A; Fully Nested ANOVA, $p<0.0001$). No differences were found in the total concentration of photosynthetic pigments per zooxanthellae cell (Figure 3.8B; Fully Nested ANOVA, $p=0.419$). No differences in total photosynthetic pigments per host tissue area (Fully Nested ANOVA, $p=0.634$) and total concentration of photosynthetic pigments per zooxanthellae cell (Fully Nested ANOVA, $p=0.966$) were found between replicates among treatments. Nevertheless, colonies of *A. cervicornis* located under the two treatments showed a slight darkening in coloration by the end of the experiment. Both the control and the Hyzod® colonies showed a slight decrease after one month of exposure; yet, by the end of the experiment all the colonies had increased their correspondent pigment concentrations. Signs of bleaching were not seen in any of the control or treated colonies.

Figure 3.9 shows a representative chromatogram identifying major photosynthetic pigments found in both species. Chlorophyll *a* concentration per coral tissue area of *A. cervicornis* did not change during the first month of exposure in either treatments or control colonies. Similarly, a significant change in chlorophyll *a* per zooxanthellae cell in *A. cervicornis* was not evident until the end of the experiment (Figure 3.10). Accessory pigments such as chlorophyll $c_2$ and peridinin showed similar fluctuations during the experiment with a significant increase by the end of the experiment (Figures 3.11-3.12). Tables 3.1 and 3.2 (Appendix II) show the concentration of all photosynthetic pigments

detected in *A. cervicornis* during the UV-E Experiment per coral tissue area and per zooxanthellae cell, respectively.

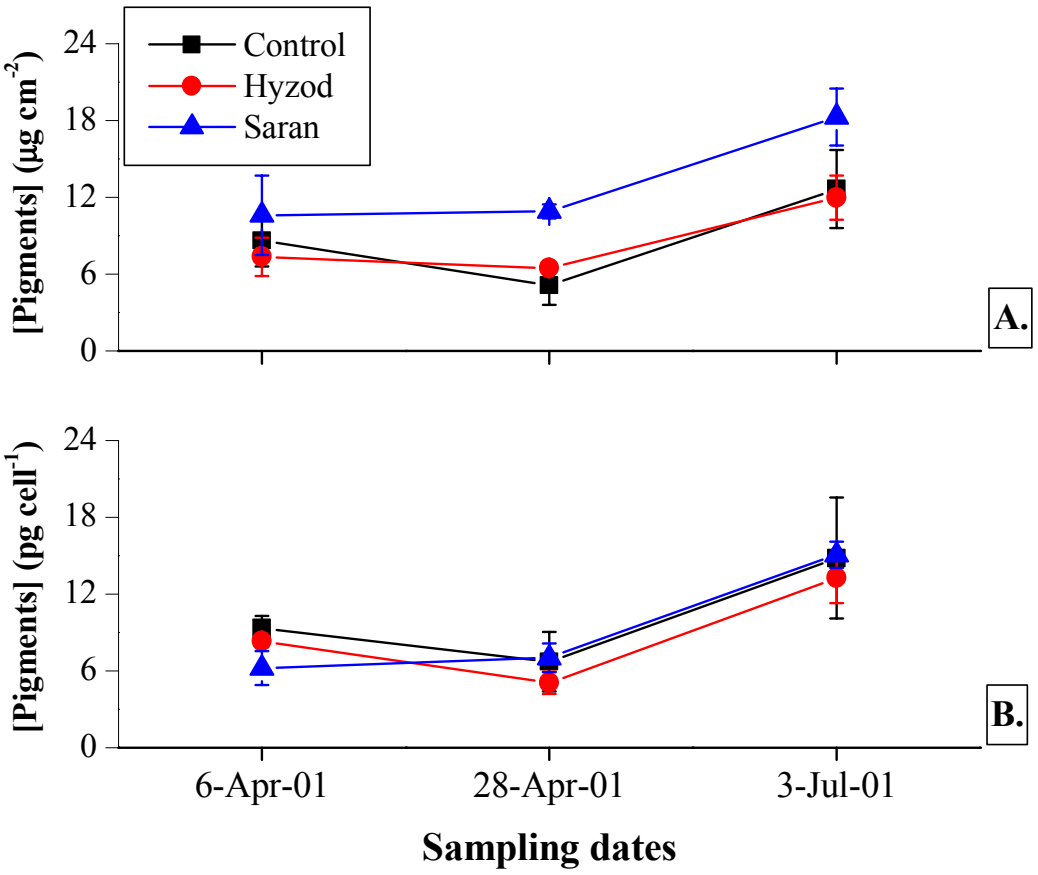


Figure 3.8. Total concentration of photosynthetic pigments (chlorophylls, carotenoids and xanthophylls) normalized to A) coral tissue area and B) zooxanthellae density in *A. cervicornis* during the UV-E Experiment. Vertical lines denote ±1SD.

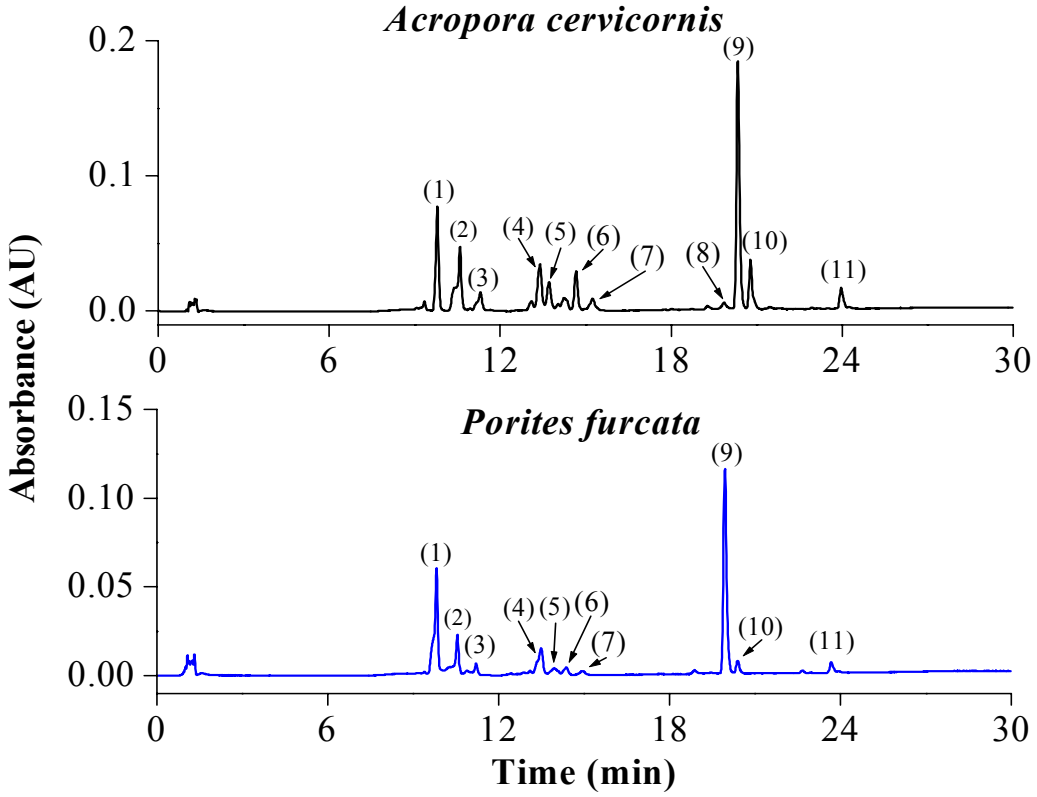


Figure 3.9. Representative chromatogram showing major photosynthetic pigments found in both species. (1) = chlorophyll $c_2$; (2) = peridinin; (3) = P-457; (4) = diadinoxanthin; (5) = diadinochromes (I or II); (6) = zeaxanthin; (7) = diatoxanthin; (8) = chlorophyll *a* allomer; (9) = chlorophyll *a*; (10) = chlorophyll *a* epimer; and (11) = β,β-carotene.

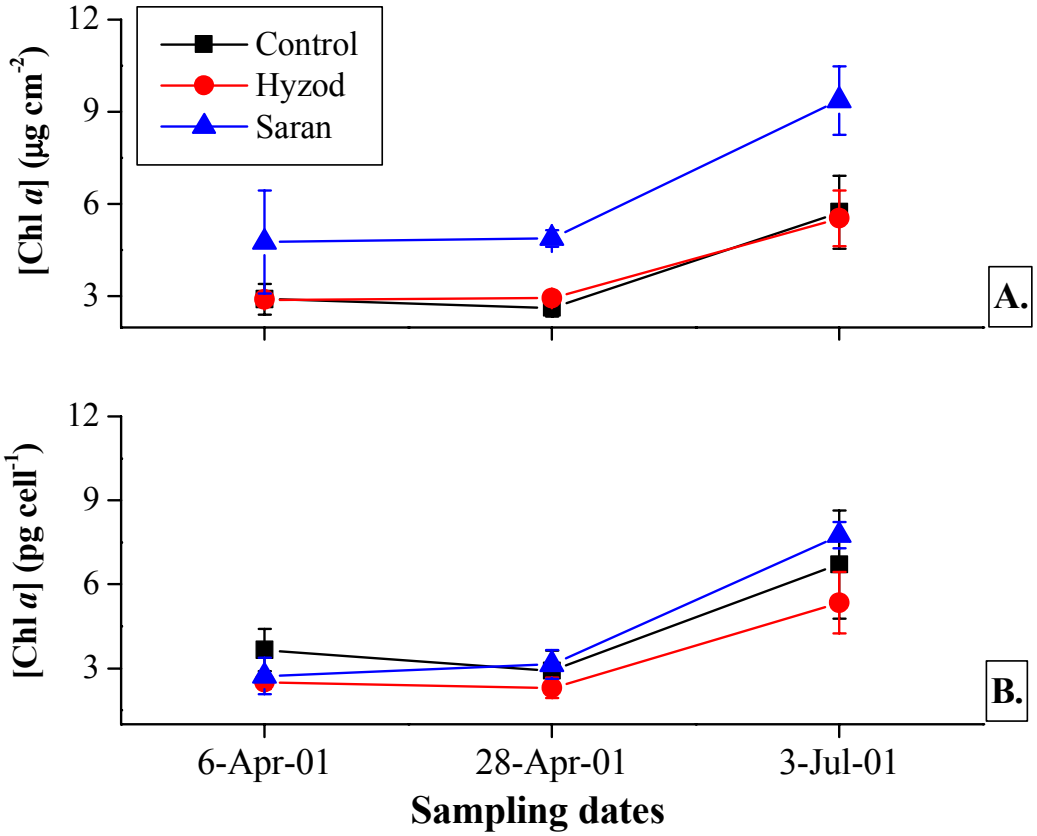


Figure 3.10. Chlorophyll *a* concentration normalized to A) coral tissue area and B) zooxanthellae density in *A. cervicornis* during the UV-E Experiment. Vertical lines denote ±1SD.

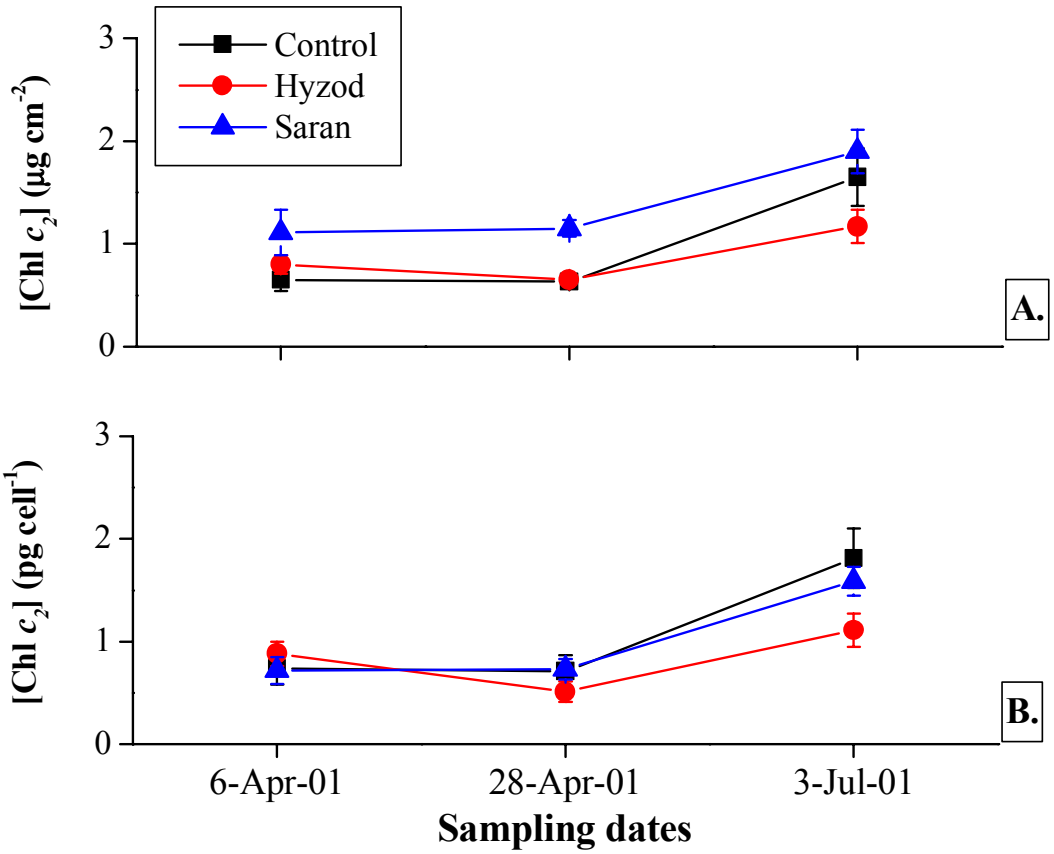


Figure 3.11. Chlorophyll $c_2$ concentration normalized to A) coral tissue area and B) zooxanthellae density in *A. cervicornis* during the UV-E Experiment. Vertical lines denote ±1SD.

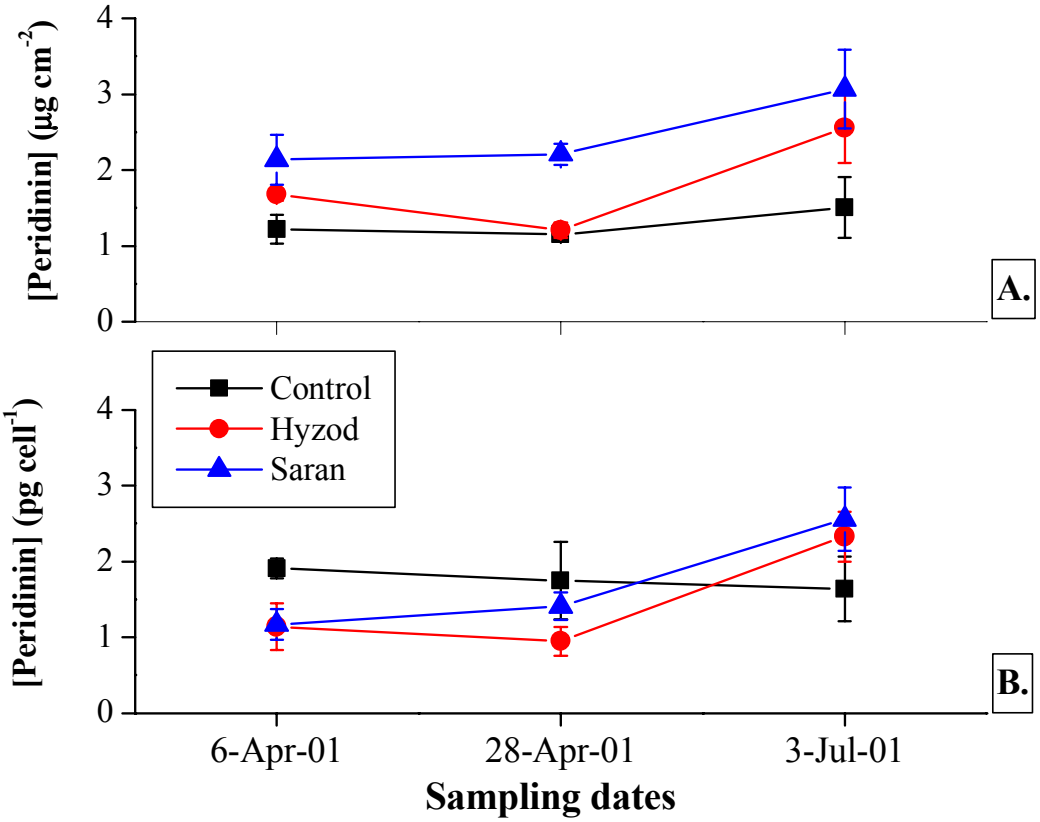


Figure 3.12. Peridinin concentration normalized to A) coral tissue area and B) zooxanthellae density in *A. cervicornis* during the UV-E Experiment. Vertical lines denote ±1SD.

In *Porites furcata*, a significant increase in total photosynthetic pigments concentration both normalized to host tissue area (Figure 3.13A; Fully Nested ANOVA, $p<0.0001$), and per zooxanthellae cell (Figure 3.13B; Fully Nested ANOVA, $p<0.0001$) was found. No differences in total photosynthetic pigments per host tissue area (Fully Nested ANOVA, $p=0.887$) and total concentration of photosynthetic pigments per zooxanthellae cell (Fully Nested ANOVA, $p=0.090$) were found between replicates among treatments. The colonies under the Saran® treatment contained significantly

higher amounts of total photosynthetic pigments than both the control and Hyzod® colonies. As in *A. cervicornis*, no signs of bleaching were observed in the treated colonies of *P. furcata* during the experiment. Also, a darkening of the colonies coloration was observed in the Saran and Hyzod colonies by the end of the experiment. The control colonies maintained a similar coloration during the experimental time.

Colonies of *P. furcata* located either under the Hyzod® or Saran® treatments showed a significant increase in chlorophyll *a*, chlorophyll $c_2$, and peridinin (individual pigments concentrations analyzed by Fully Nested ANOVA, $p<0.0001$, $p<0.0001$, and $p<0.001$, respectively) by the end of the experiment (Figures 3.14-3.16). Tables 3.3 and 3.4 (Appendix II) show the concentration of all photosynthetic pigments detected in *P. furcata* during the UV-E Experiment per coral tissue area and per zooxanthellae cell, respectively.

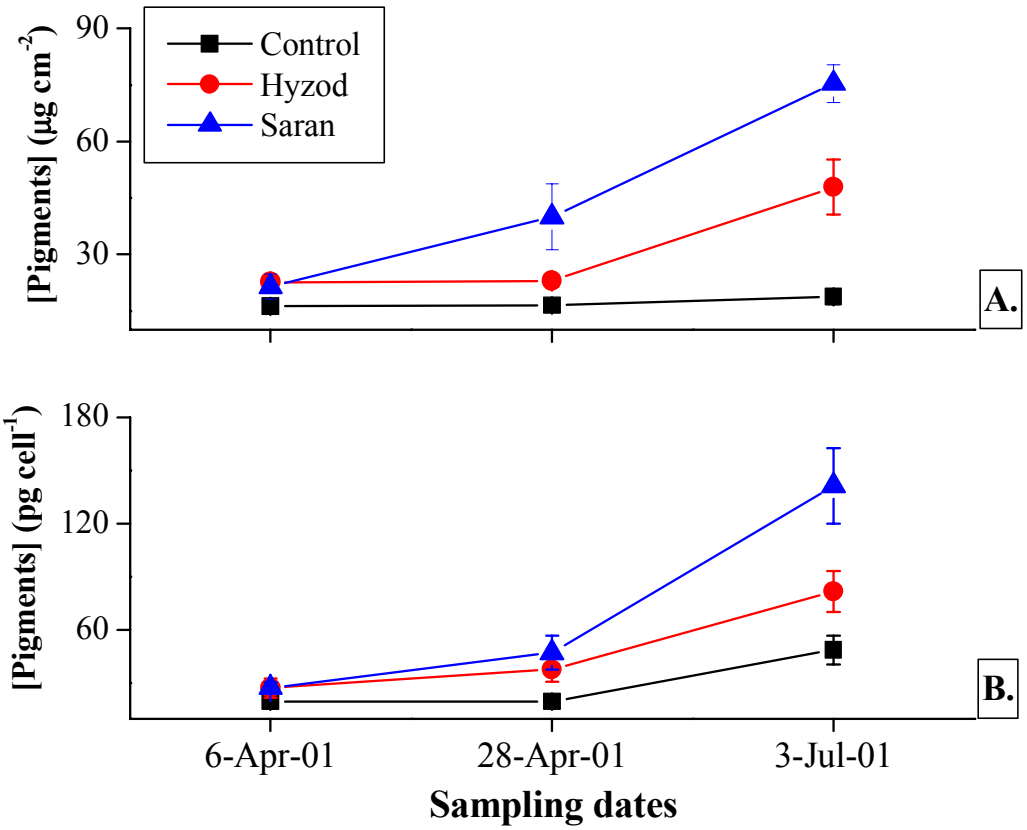


Figure 3.13. Total concentration of photosynthetic pigments (chlorophylls, carotenoids and xanthophylls) normalized to A) coral tissue area and B) zooxanthellae density in *P. furcata* during the UV-E Experiment. Vertical lines denote ±1SD.

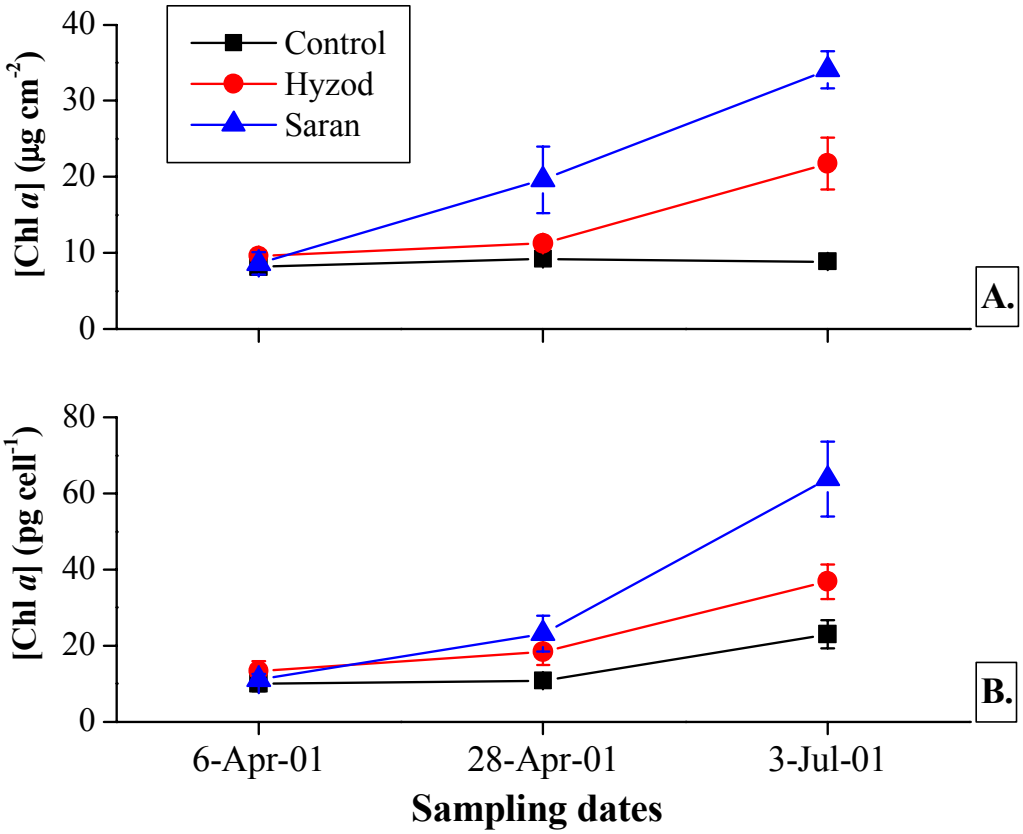


Figure 3.14. Chlorophyll *a* concentration normalized to A) coral tissue area and B) zooxanthellae density in *P. furcata* during the UV-E Experiment. Vertical lines denote ±1SD.

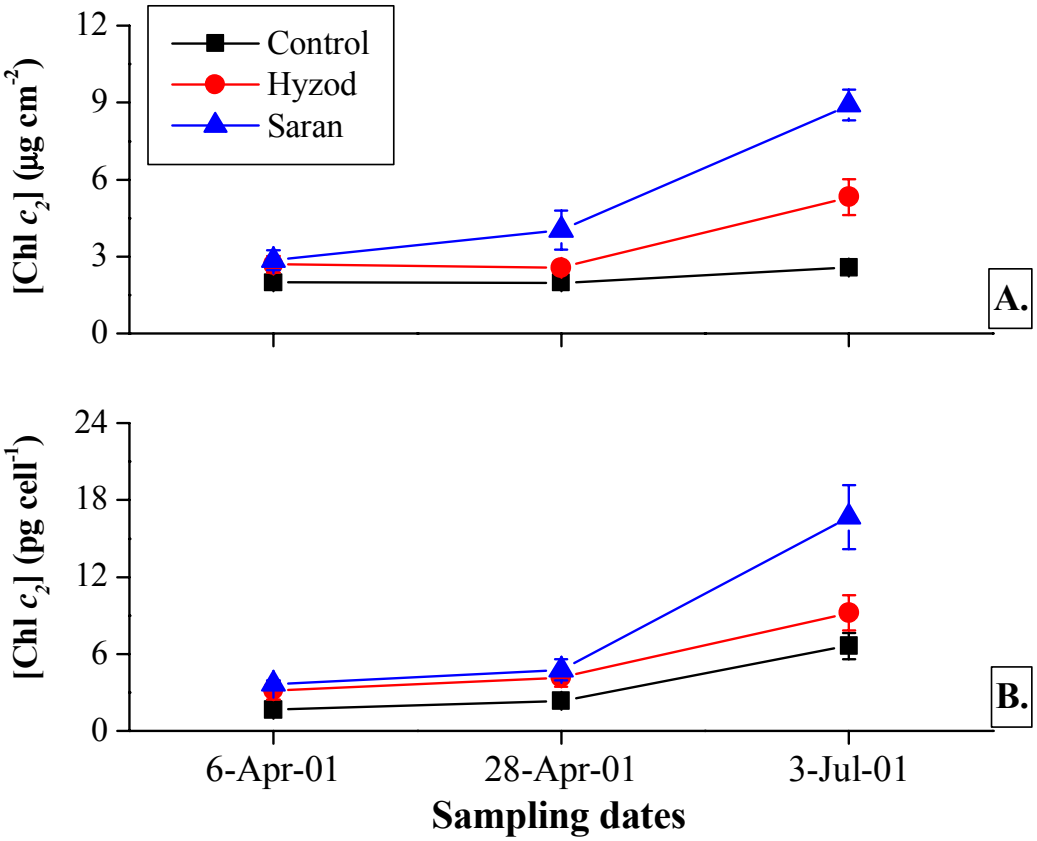


Figure 3.15. Chlorophyll $c_2$ concentration normalized to A) coral tissue area and B) zooxanthellae density in *P. furcata* during the UV-E Experiment. Vertical lines denote ±1SD.

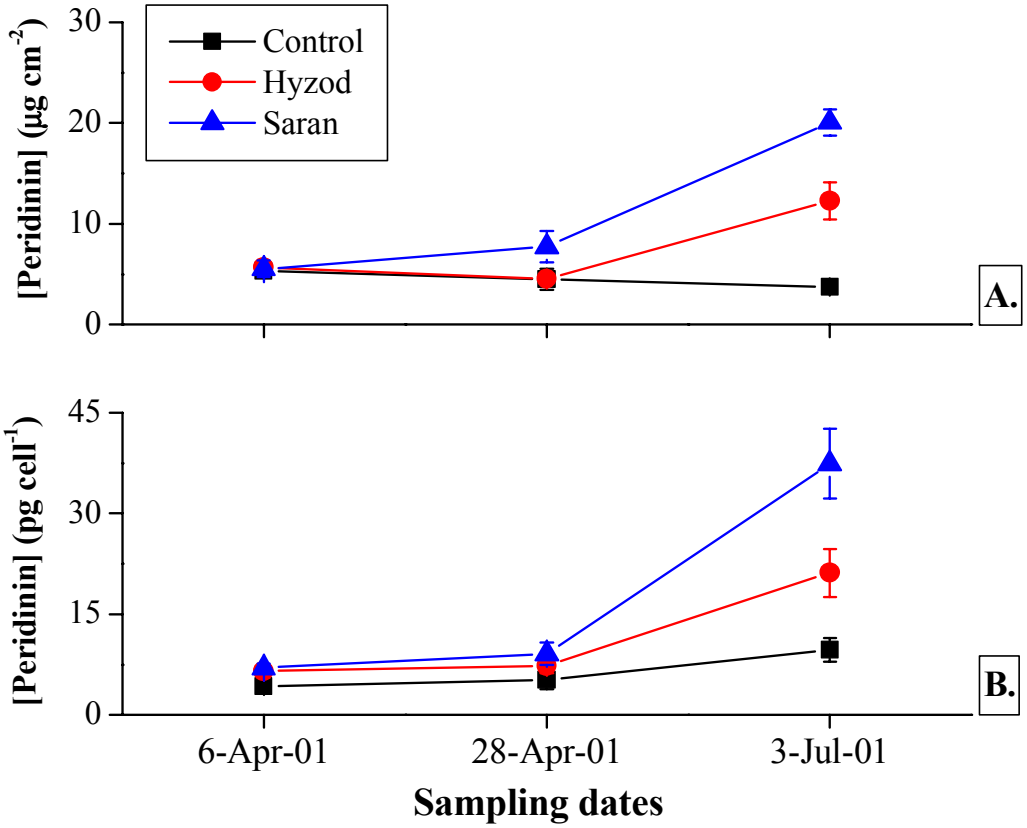


Figure 3.16. Peridinin concentration normalized to A) coral tissue area and B) zooxanthellae density in *P. furcata* during the UV-E Experiment. Vertical lines denote ±1SD.

**Zooxanthellae**

Zooxanthellae concentrations (normalized to host tissue area) within the tissues of both species were not significantly different among treatments (*A. cervicornis*, Fully Nested ANOVA, p=0.169; *P. furcata*, Fully Nested ANOVA, p=0.142). Nevertheless, there is a tendency of increasing the number of zooxanthellae with a decrease in PAR and UVR levels (Figure 3.17) in both species. No differences in zooxanthellae density normalized to host tissue area in both species [Fully Nested ANOVA, (*A. cervicornis*, p=0.741; *P. furcata*, p=0.552)] were found between replicates among treatments.

The two species responded differently relative to the mitotic index (percent of cells dividing at any one particular time) of their respective zooxanthellae. *A. cervicornis* showed an inverse significant (Fully Nested ANOVA, p=0.028) relationship in the mitotic index with decreasing UVR and PAR levels (Figure 3.18). A Tukey Test indicated that only the Hyzod® and control colonies were significantly different (p=0.022), and no difference was found either between the control and Saran colonies (p=0.057) or the Hyzod® and Saran® colonies (p=0.840). *P. furcata* showed a positive, but not significant (Fully Nested ANOVA, p=0.259) relationship between the zooxanthellae cells mitotic index and the decreased UVR and PAR levels reaching the colonies (Figure 3.18). No differences in zooxanthellae mitotic index in both species [Fully Nested ANOVA, (*A. cervicornis*, p=0.539; *P. furcata*, p=0.245)] were found between replicates among treatments.

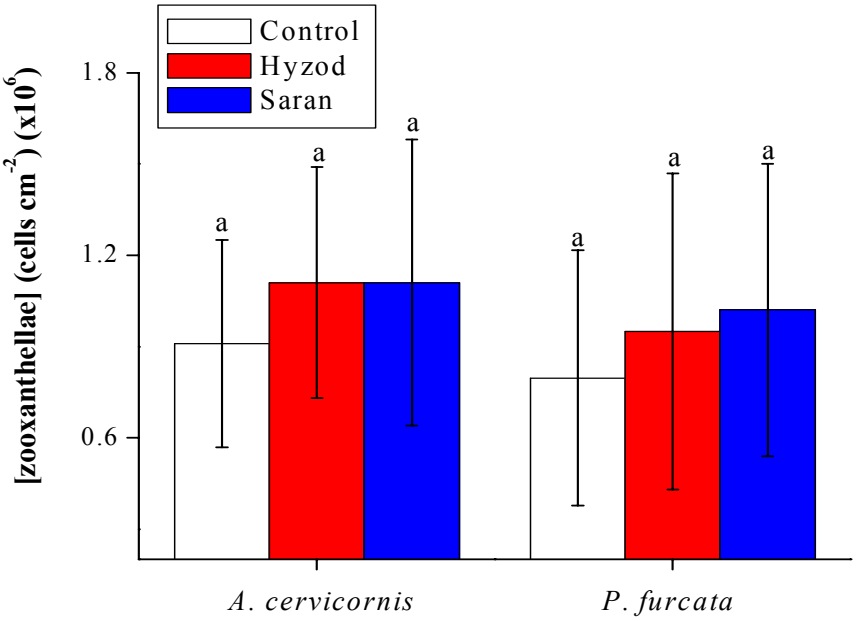


Figure 3.17. Zooxanthellae densities (normalized to coral tissue area) present in both species at the end of the UV-E Experiment. Vertical lines denote ±1SD.

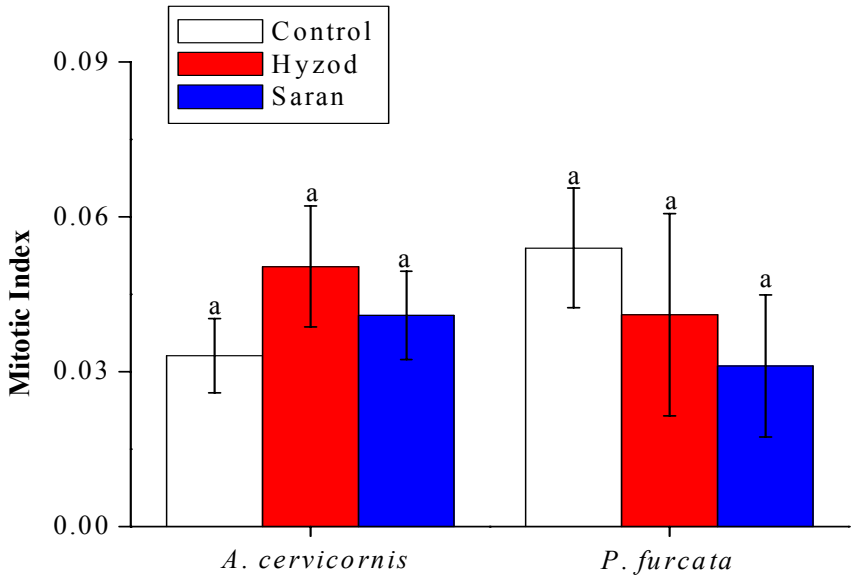


Figure 3.18. Mitotic Index (MI) of the zooxanthellae present in both species at the end of the UV-E Experiment. MI is expressed as the amount of cells dividing/total amount of cells present in a given sample. Vertical lines denote ±1SD.

## UV-S Experiment

### Mycosporine-like Amino Acids (MAA's)

Colonies of *Acropora cervicornis* normally living at 1m depth at San Cristóbal Reef contained 40-65 times higher total MAA's than those living at 20m at the shelf edge with a steady increase as summertime approached. There was a significant difference among transplants (Fully Nested ANOVA, $p<<0.0001$). Those colonies transplanted to deeper reef zones drastically reduced their respective MAA's concentrations either as a whole (Figure 3.19) or individually (Figures 3.20-3.21). Colonies transplanted from 1m and 8m to 20m showed no significant difference in total MAA's compared to the controls at 20m (Fully Nested ANOVA, $p=0.994$). The colonies transplanted to shallower reef zones, despite a severe bleaching event in those colonies transplanted from 20m to 1m depth (see below), showed an approximately 40-times increase by the first month of exposure and 88-times higher total MAA's by the end of the experiment compared to control colonies at 20m. Those colonies transplanted from 8m and 20m to 1m showed a significant increase in total MAA's compared to the controls at 1m (Fully Nested ANOVA, $p<0.001$). The relationship between the total concentration of MAA's and the levels of total UVR measured at 20m (Old Buoy) vs.1m depth (San Cristóbal Reef) is shown in Figure 3.22. Total MAA's were significantly higher in colonies living at 1m vs. colonies living at 20m regardless of their original site (i.e., controls or transplants) (t-test, $p<0.0001$). There were no significant differences in total MAA's between colonies among replicates of the same treatment or between replicates of the same treatment (Fully Nested ANOVA, $p=0.992$).

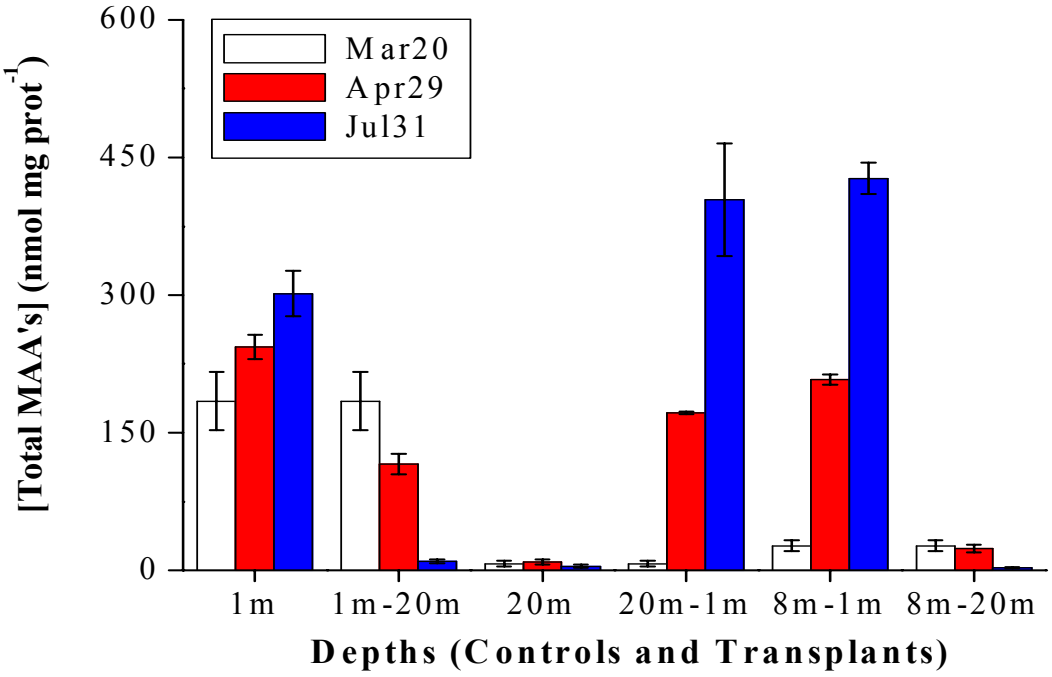


Figure 3.19. Total MAA's concentration in *A. cervicornis* (nmol mg protein$^{-1}$) measured during the UV-S Experiment. Legend: 1m = control colonies at the back-reef area of San Cristóbal Reef; 20m = control colonies at the Old Buoy shelf edge area; other columns refer to original vs. transplanted depth. Vertical lines denote ±1SD.

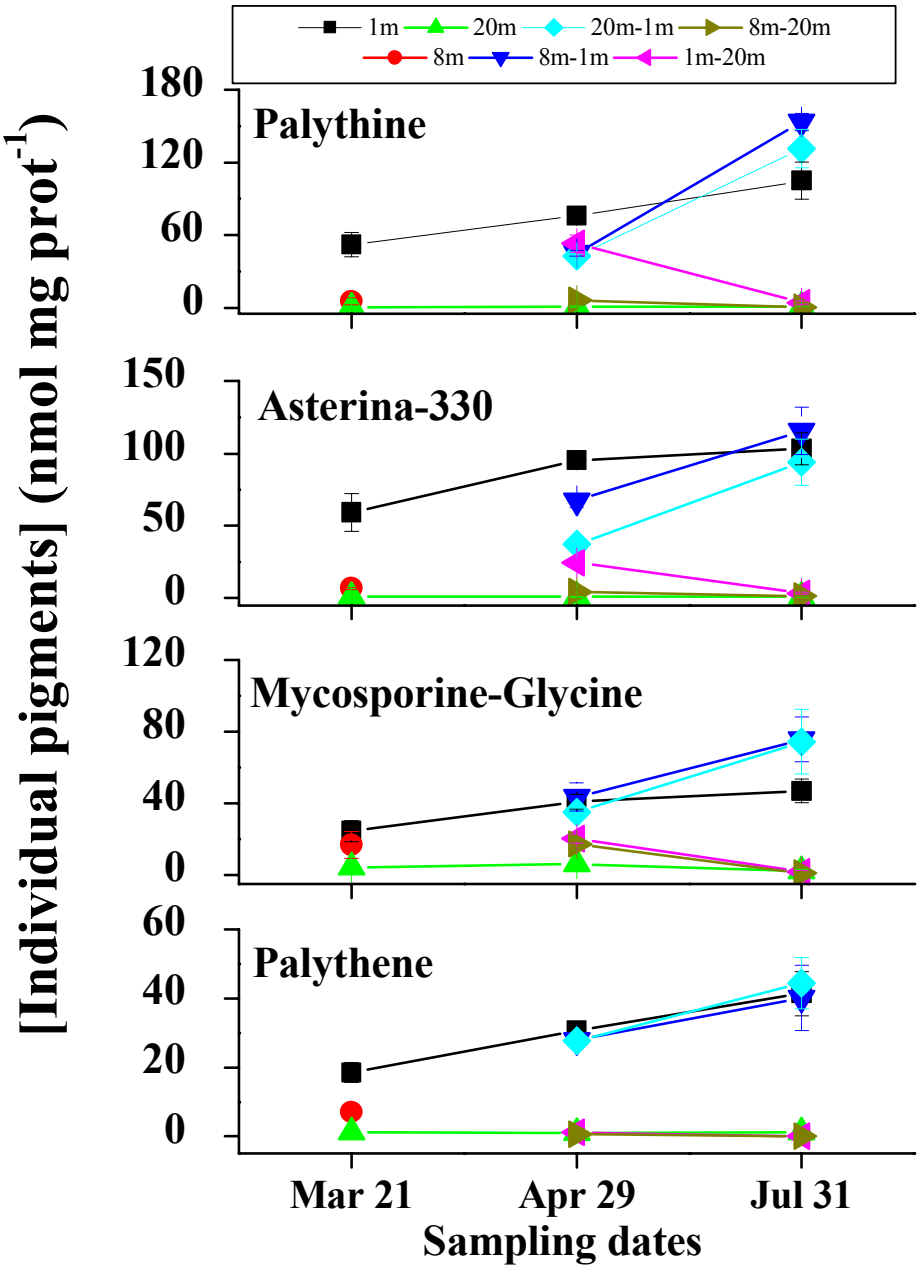


Figure 3.20. Concentration of palythine (λmax = 320nm), asterina-330 (λmax = 330nm), mycosporine-glycine (λmax = 310nm), and palythene (λmax = 360nm) in *A. cervicornis* during the UV-S Experiment. Legend: 1m = control colonies at the back-reef area of San Cristóbal Reef; 20m = control colonies at the Old Buoy shelf edge area; other columns refer to original vs. transplanted depth. Vertical lines denote ±1SD.

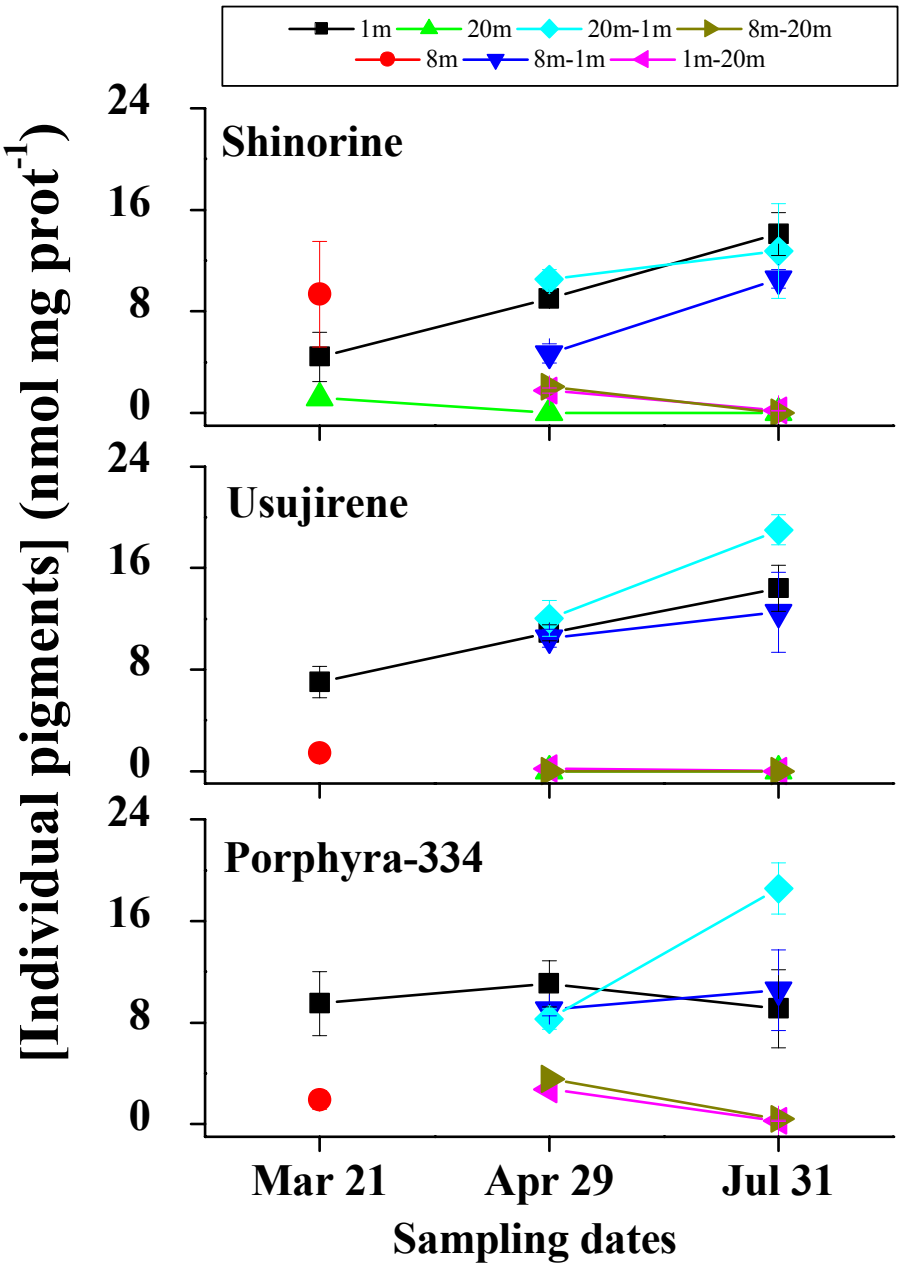


Figure 3.21. Concentration of shinorine (λmax = 333nm), usujirene (λmax = 357nm), and porphyra-334 (λmax = 334nm) in *A. cervicornis* during the UV-S Experiment. Legend: 1m = control colonies at the back-reef area of San Cristóbal Reef; 20m = control colonies at the Old Buoy shelf edge area; other columns refer to original vs. transplanted depth. Vertical lines denote ±1SD.

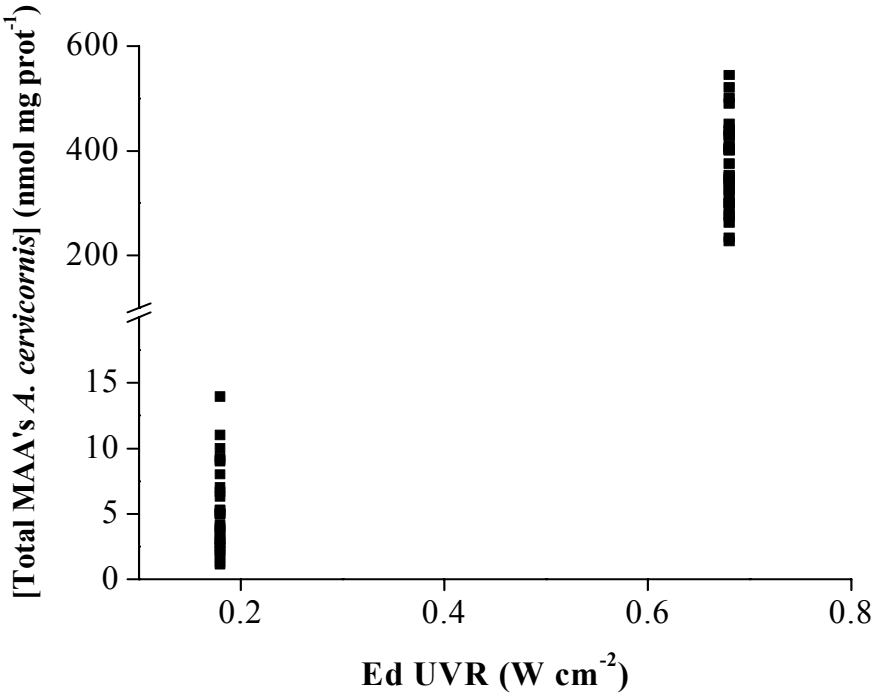


Figure 3.22. Relationship between total UVR measured at the shelf edge site (Old Buoy, 20m) and San Cristóbal Reef (1m), and total concentration of MAA's found in *A. cervicornis*.

The significant increase in total MAA's in colonies transplanted from the shelf edge to San Cristóbal Reef suggests a virtually immediate response to higher UVR levels for host and symbionts protection. While the colonies transplanted to deeper areas eliminated their longer wavelength UV-absorbent MAA's (i.e., palythene and usujirene), the colonies transplanted to 1m showed a higher increase in all MAA's especially those absorbing near the UV-B region (i.e., palythine).

Colonies of *Porites furcata* subjected to artificially enhanced UVR levels showed a significant increase in total MAA concentration (Figure 3.23; Fully Nested ANOVA, $p<0.0001$) relative to the control colonies and individually (Figures 3.24-3.25). There were no significant differences in total MAA's between colonies among replicates of the

same treatment or between replicates of the same treatment (Fully Nested ANOVA, p=0.998). Total MAA concentration showed an exponential increase with increased levels of UVR (Figure 3.26). Those colonies exposed to reduced UVR levels under the Hyzod® reacted similarly to those colonies exposed to similar conditions in the field during the UV-E Experiment. *P. furcata* colonies contained higher amounts of mycosporine-glycine ($\lambda_{max}$ = 310nm) than any other MAA (Figures 3.24-3.25) after exposure to the Q-Panel lamps (313nm peak maxima) showing a direct response to increased UV-B doses.

In general, while both species contained similar MAA's, they differ in the production preference of individual MAA's. *A. cervicornis* symbionts prefer to produce more pigments that absorb near or in the UV-A region (i.e., palythine and asterina-330). On the other hand, *P. furcata* colonies preferably produced more pigments that absorb into the UV-B region (i.e., mycosporine-glycine).

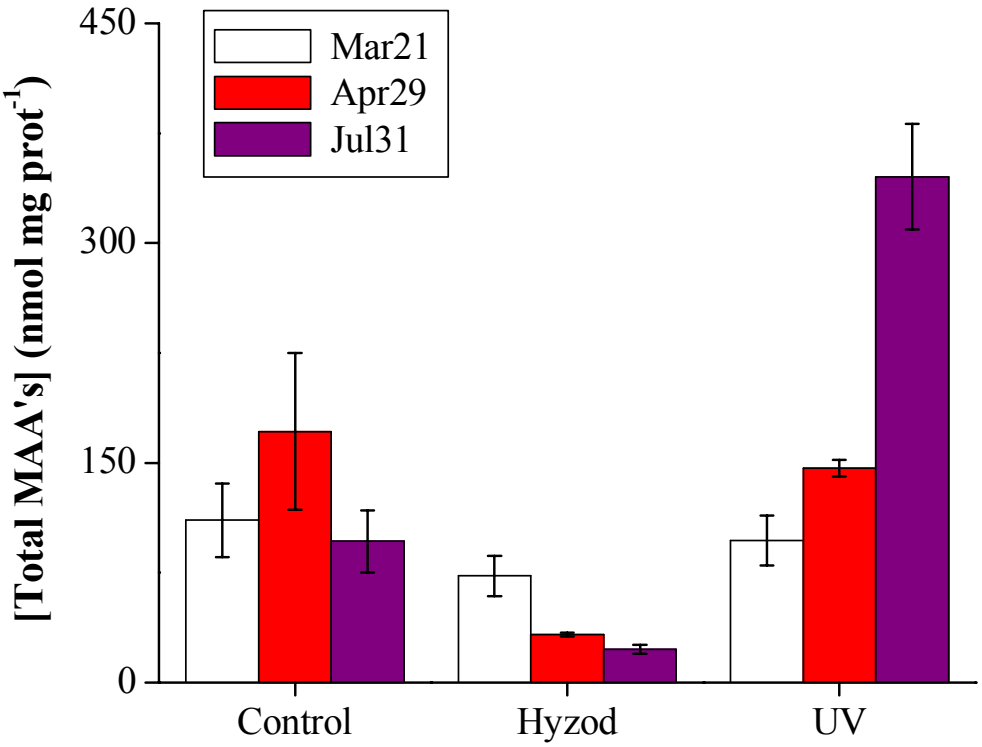


Figure 3.23. Total MAA's concentration in *P. furcata* (nmol mg protein$^{-1}$) measured during the UV-S Experiment. Vertical lines denote ±1SD.

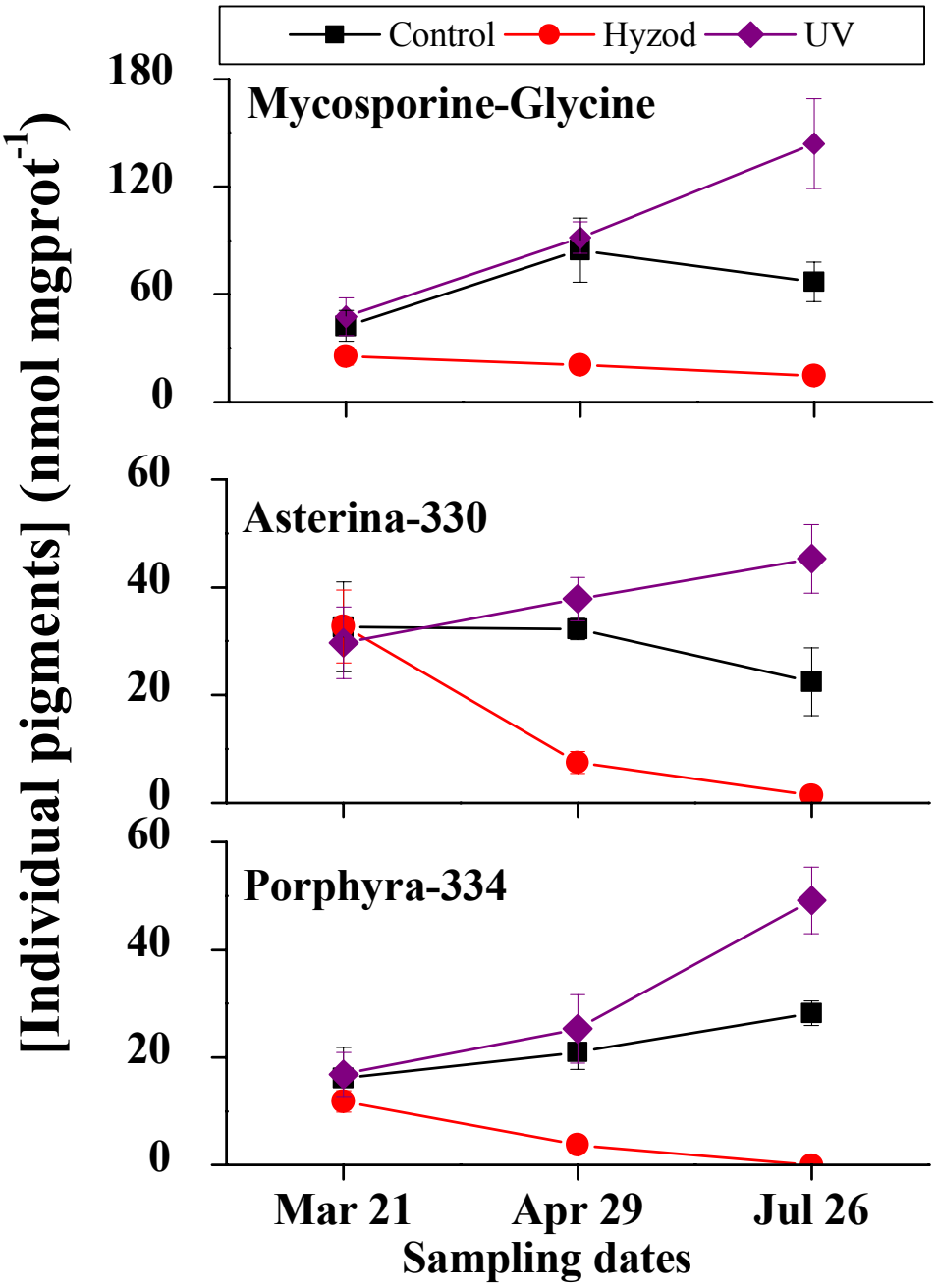


Figure 3.24. Concentration of mycosporine-glycine (λmax = 310nm), asterina-330 (λmax = 330nm), and porphyra-334 (λmax = 334nm) in *P. furcata* during the UV-S Experiment. Vertical lines denote ±1SD.

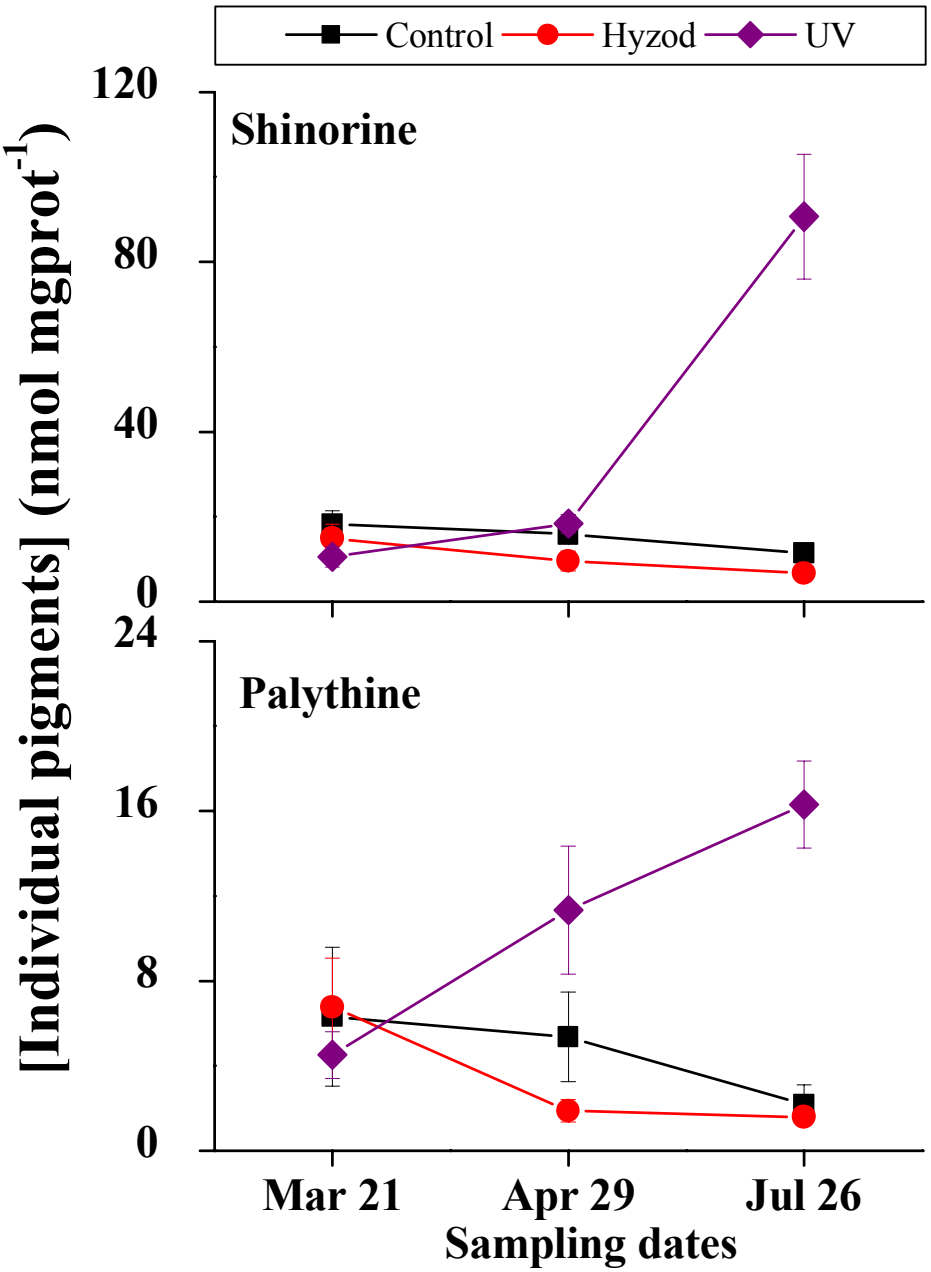


Figure 3.25. Concentration of shinorine (λmax = 333nm) and palythine (λmax = 320nm) in *P. furcata* during the UV-S Experiment. Vertical lines denote ±1SD.

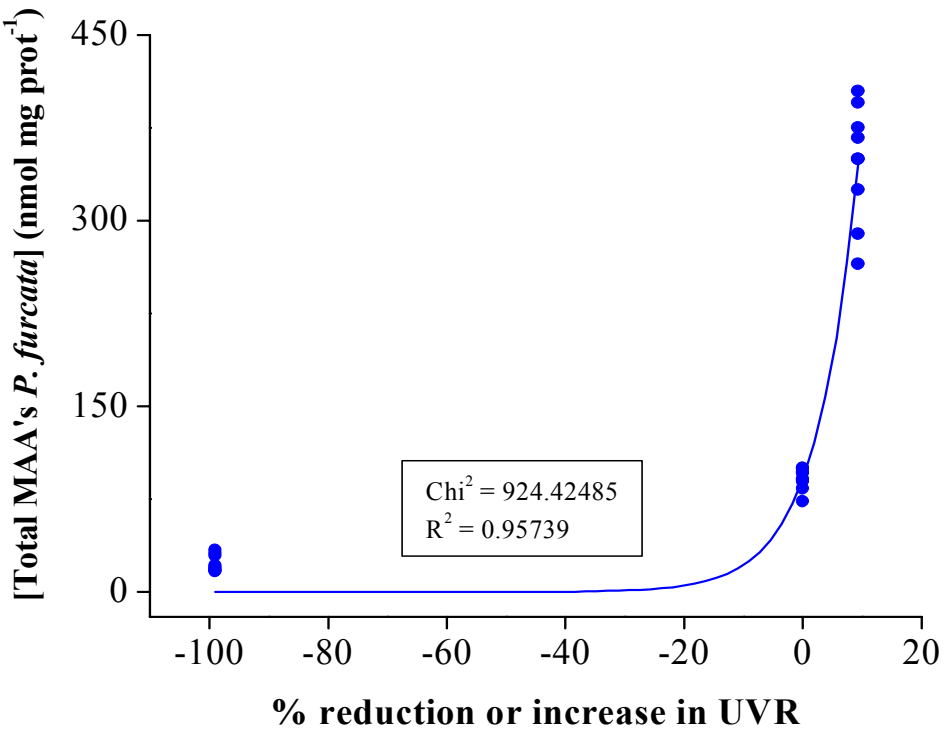


Figure 3.26. Exponential relationship between UVR levels and total concentration of MAA's in *P. furcata*.

**Photosynthetic pigments**

The total amount of photosynthetic pigments in *Acropora cervicornis* varied significantly among treatments (e.g., transplants to different depths) (Fully Nested ANOVA, $p<0.0001$). No differences in total photosynthetic pigments per host tissue area (Fully Nested ANOVA, $p=0.081$) and total concentration of photosynthetic pigments per zooxanthellae cell (Fully Nested ANOVA, $p=0.562$) were found between replicates among treatments. Colonies of *A. cervicornis* transplanted from 20m to 1m depth showed immediate bleaching signs just one day after the transplant. This was reflected in a significant decrease in total photosynthetic pigments per coral tissue area (Figure 3.27A) and to a lesser degree per zooxanthellae cells (Figure 3.27B). Similarly, colonies

transplanted to shallower reef zones showed decreased concentrations of major photosynthetic pigments (i.e., chlorophyll *a*, chlorophyll $c_2$, and peridinin) (Figures 3.28-3.30). Those colonies transplanted from shallow and intermediate reef zones to deeper areas showed an increase in major pigments concentrations. Tables 3.5-3.8 (Appendix II) show the concentrations of all photosynthetic pigments detected in *A. cervicornis* during the UV-S Experiment. While a slight increase in diatoxanthin occurred after one month of transplants relative to diadinoxanthin, suggesting a photoprotective reaction, a similar increase also occurred in the control colonies at the shelf edge; hence, the use of the xanthophyll cycle in photoprotection in both species was not demonstrated in *A. cervicornis* during this experiment.

There was a significant decrease in total photosynthetic pigments of *Porites furcata* colonies (Fully Nested ANOVA, $p<0.0001$) exposed to enhanced UVR levels relative to the control ones both per coral tissue area and per zooxanthellae cell (Figure 3.31). No differences in total photosynthetic pigments per host tissue area (Fully Nested ANOVA, $p=0.204$) and total concentration of photosynthetic pigments per zooxanthellae cell (Fully Nested ANOVA, $p=0.828$) were found between replicates among treatments. The concentration photosynthetic pigments in colonies exposed to a 99% decrease in UVR varied after three months of exposure. Major photosynthetic pigments followed the same trend (Figures 3.32-3.34). Tables 3.9 and 3.10 (Appendix II) show the concentrations of all photosynthetic pigments detected in *P. furcata* during the UV-S Experiment. Appendix II includes all the tables containing the concentration of individual photosynthetic pigments found in both species during both the UV-E and UV-S Experiments.

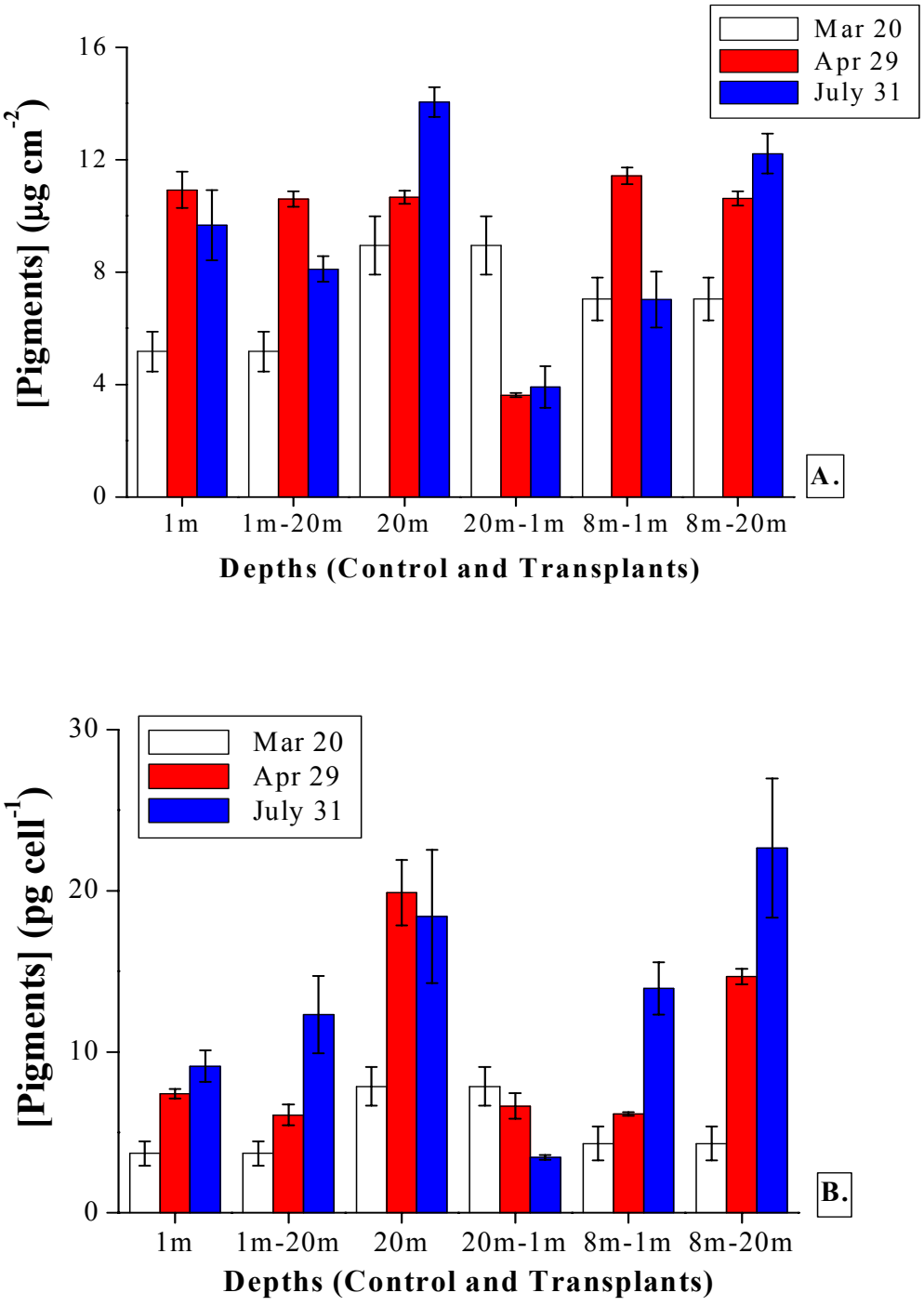


Figure 3.27. Total concentration of photosynthetic pigments (chlorophylls, carotenoids and xanthophylls) normalized to A) coral tissue area and B) zooxanthellae density in *A. cervicornis* during the UV-S Experiment. Legend: 1m = control colonies at the back-reef area of San Cristóbal Reef; 20m = control colonies at the Old Buoy shelf edge area; other columns refer to original vs. transplanted depth. Vertical lines denote ±1SD.

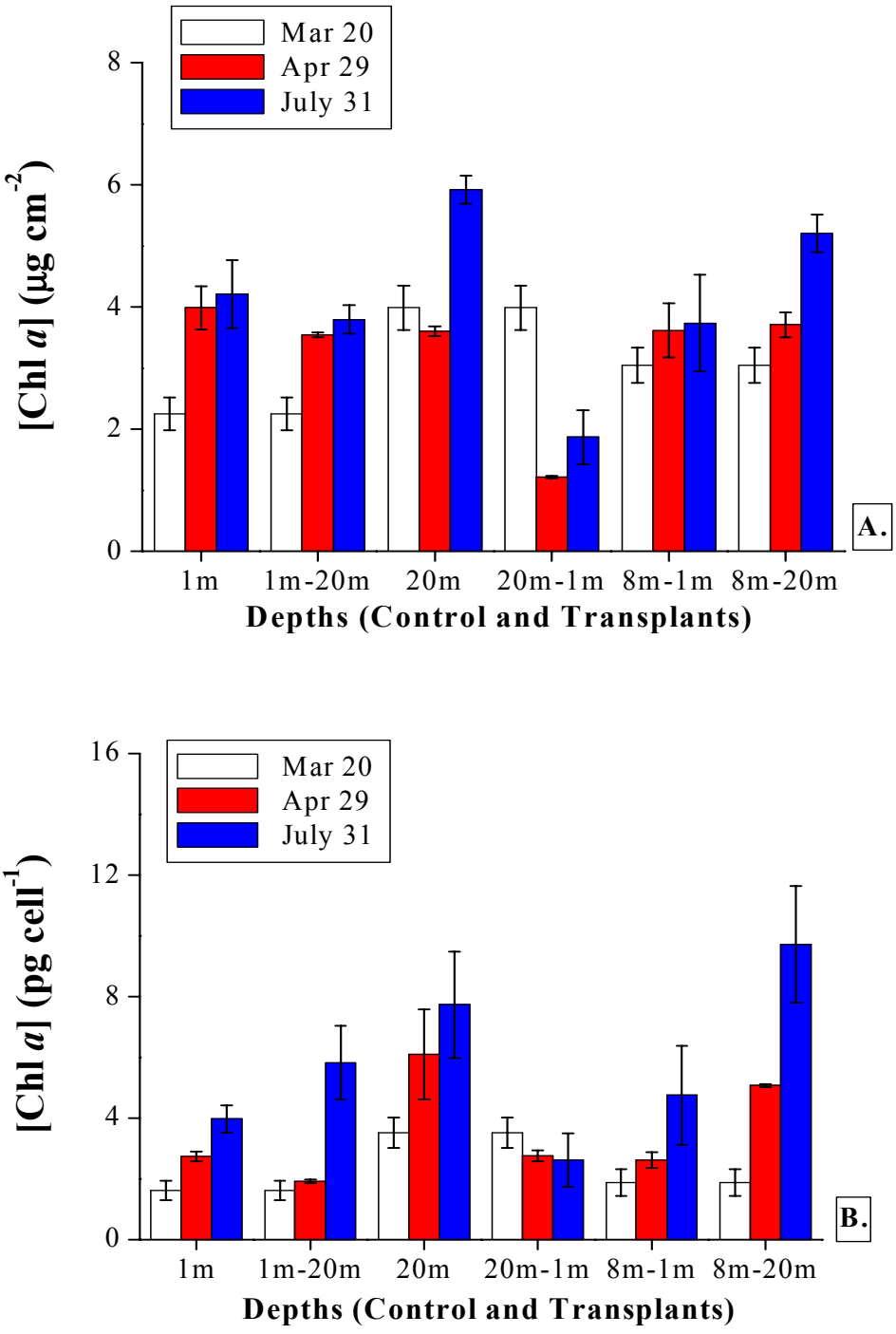


Figure 3.28. Chlorophyll *a* concentration normalized to A) coral tissue area and B) zooxanthellae density in *A. cervicornis* during the UV-S Experiment. Legend: 1m = control colonies at the back-reef area of San Cristóbal Reef; 20m = control colonies at the Old Buoy shelf edge area; other columns refer to original vs. transplanted depth. Vertical lines denote ±1SD.

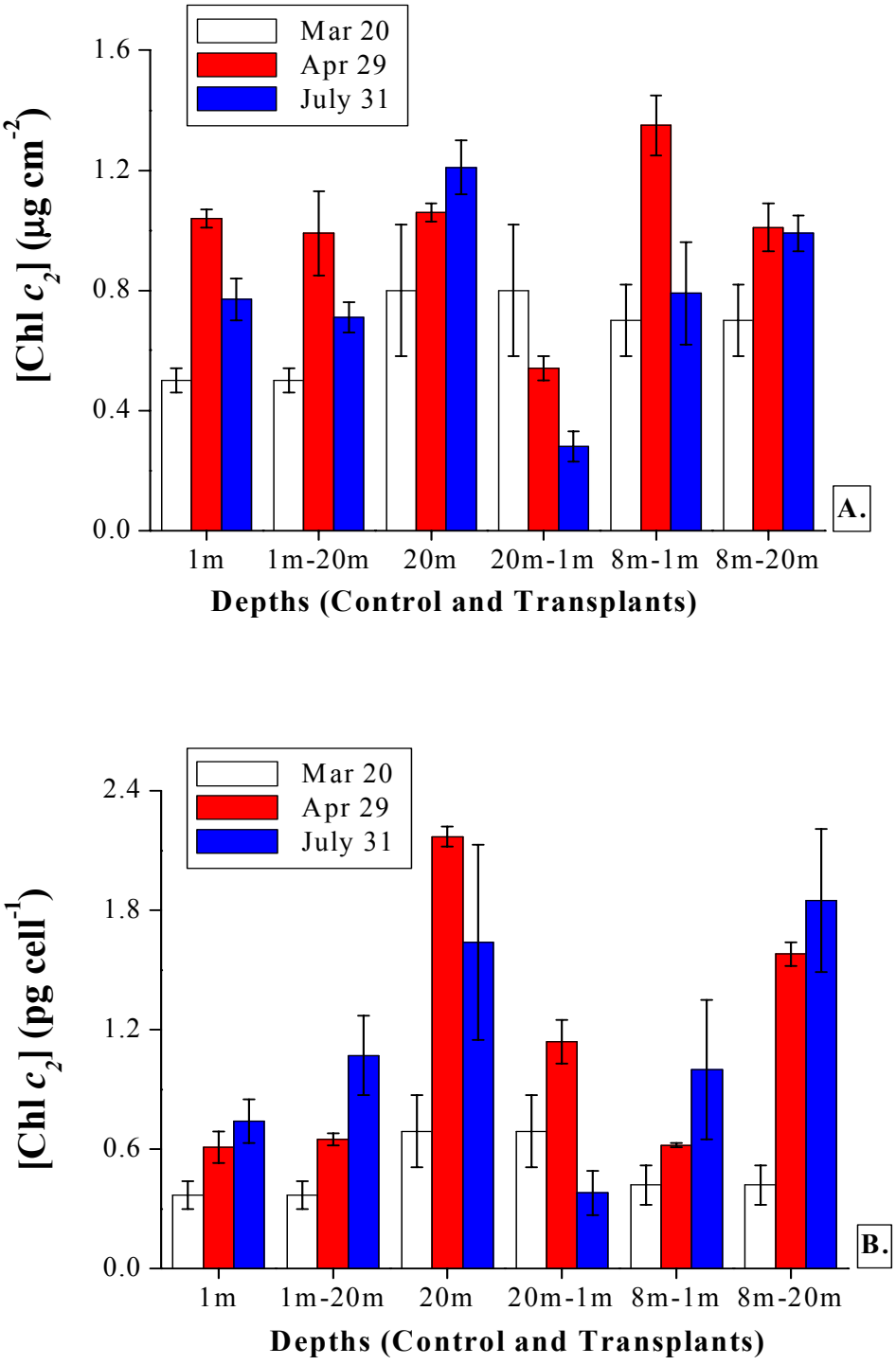


Figure 3.29. Chlorophyll $c_2$ concentration normalized to A) coral tissue area and B) zooxanthellae density in *A. cervicornis* during the UV-S Experiment. Legend: 1m = control colonies at the back-reef area of San Cristóbal Reef; 20m = control colonies at the Old Buoy shelf edge area; other columns refer to original vs. transplanted depth. Vertical lines denote ±1SD.

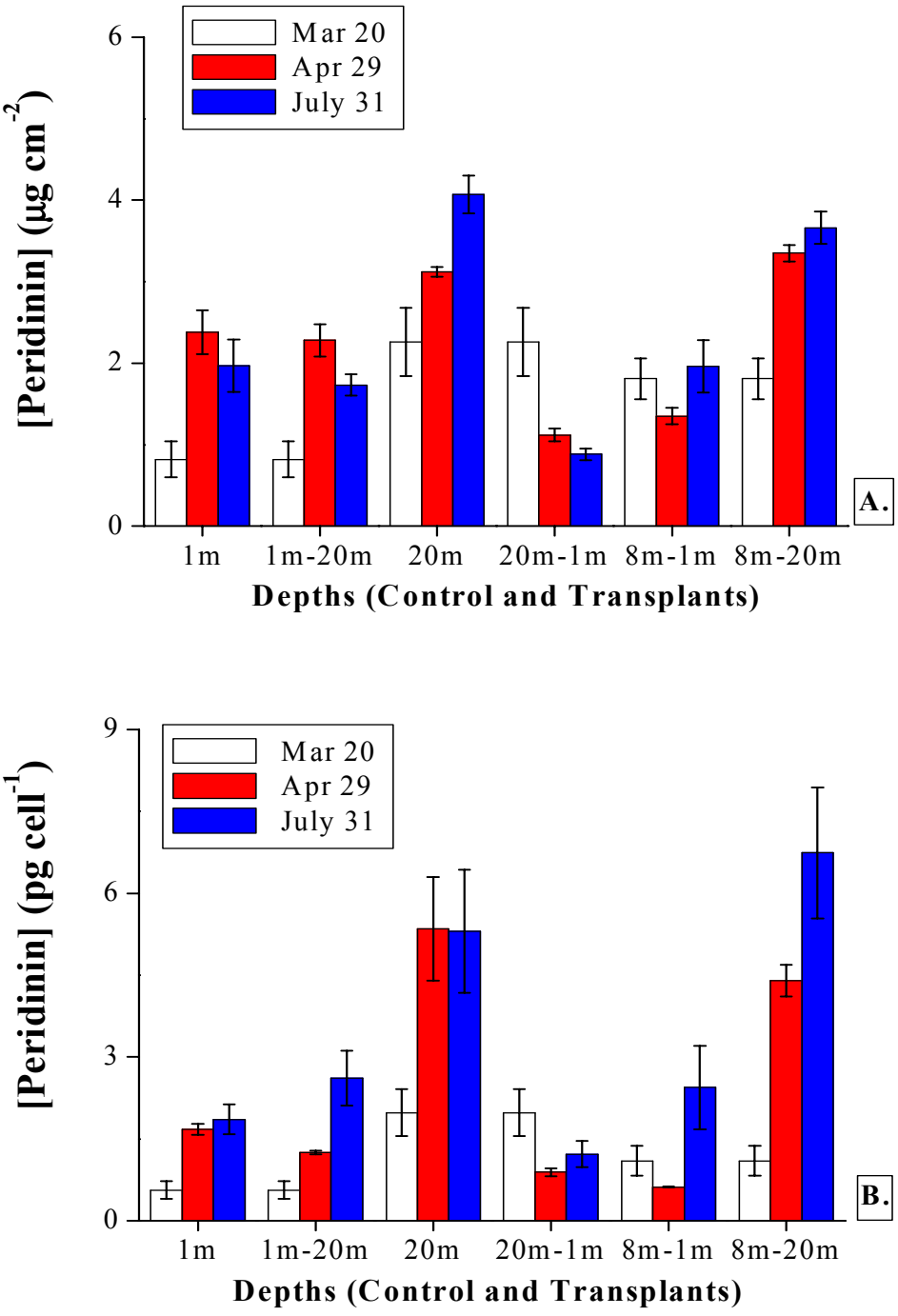


Figure 3.30. Peridinin concentration normalized to A) coral tissue area and B) zooxanthellae density in *A. cervicornis* during the UV-S Experiment. Legend: 1m = control colonies at the back-reef area of San Cristóbal Reef; 20m = control colonies at the Old Buoy shelf edge area; other columns refer to original vs. transplanted depth. Vertical lines denote ±1SD.

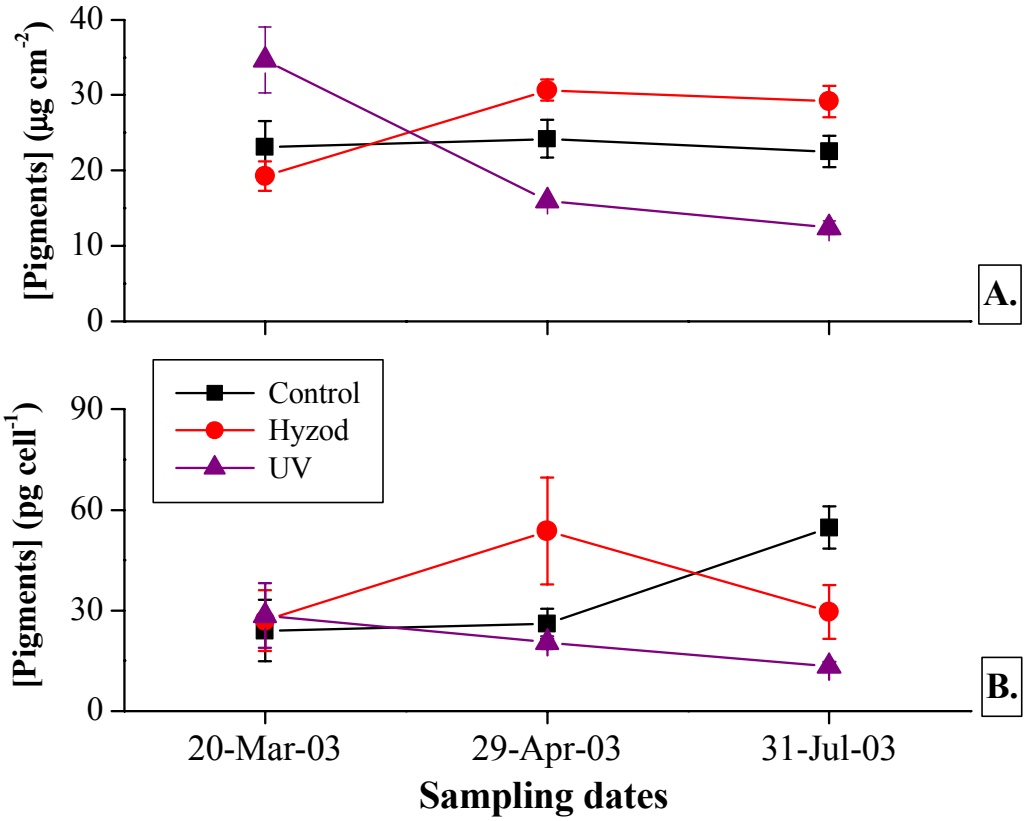


Figure 3.31. Total concentration of photosynthetic pigments (chlorophylls, carotenoids and xanthophylls) normalized to A) coral tissue area and B) zooxanthellae density in *P. furcata* during the UV-S Experiment. Vertical lines denote ±1SD.

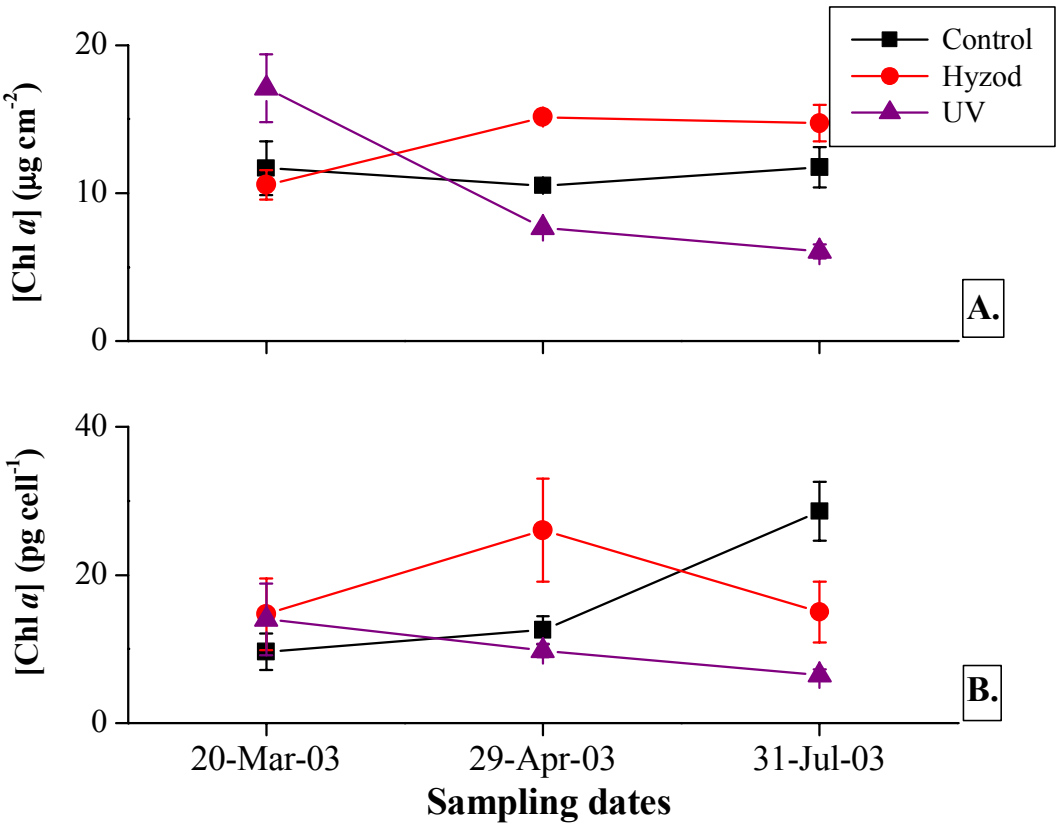


Figure 3.32. Chlorophyll *a* concentration normalized to A) coral tissue area and B) zooxanthellae density in *P. furcata* during the UV-S Experiment. Vertical lines denote ±1SD.

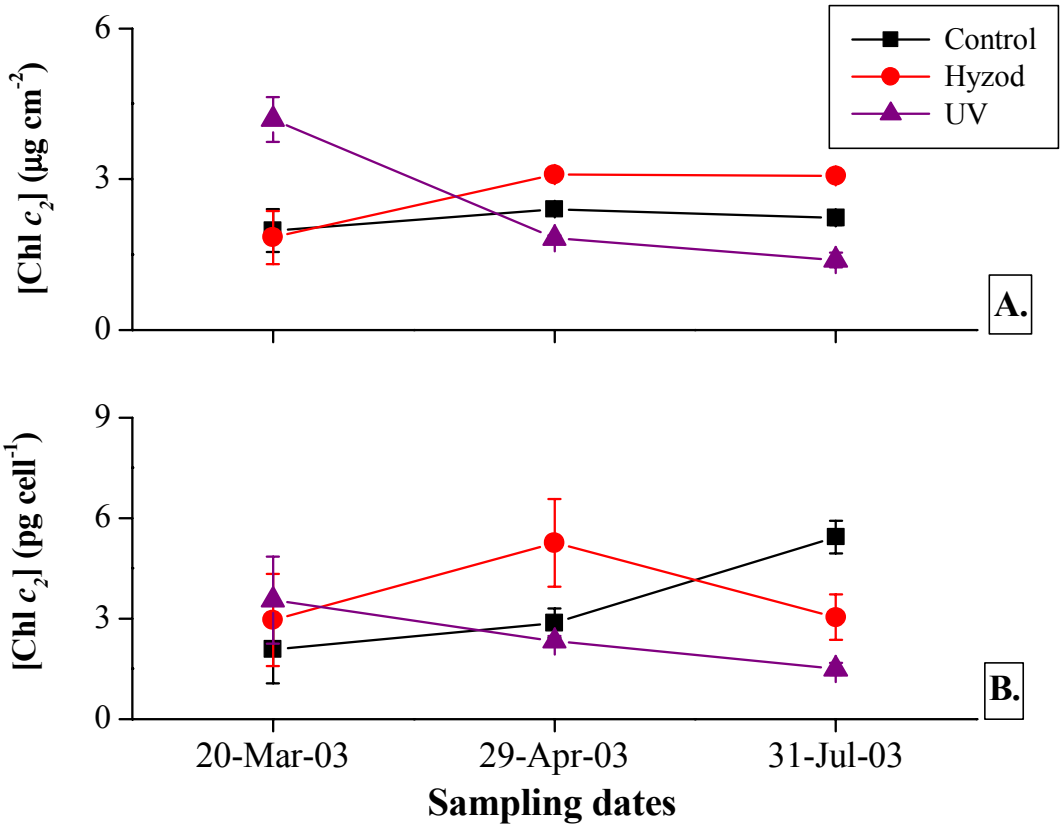


Figure 3.33. Chlorophyll $c_2$ concentration normalized to A) coral tissue area and B) zooxanthellae density in *P. furcata* during the UV-S Experiment. Vertical lines denote ±1SD.

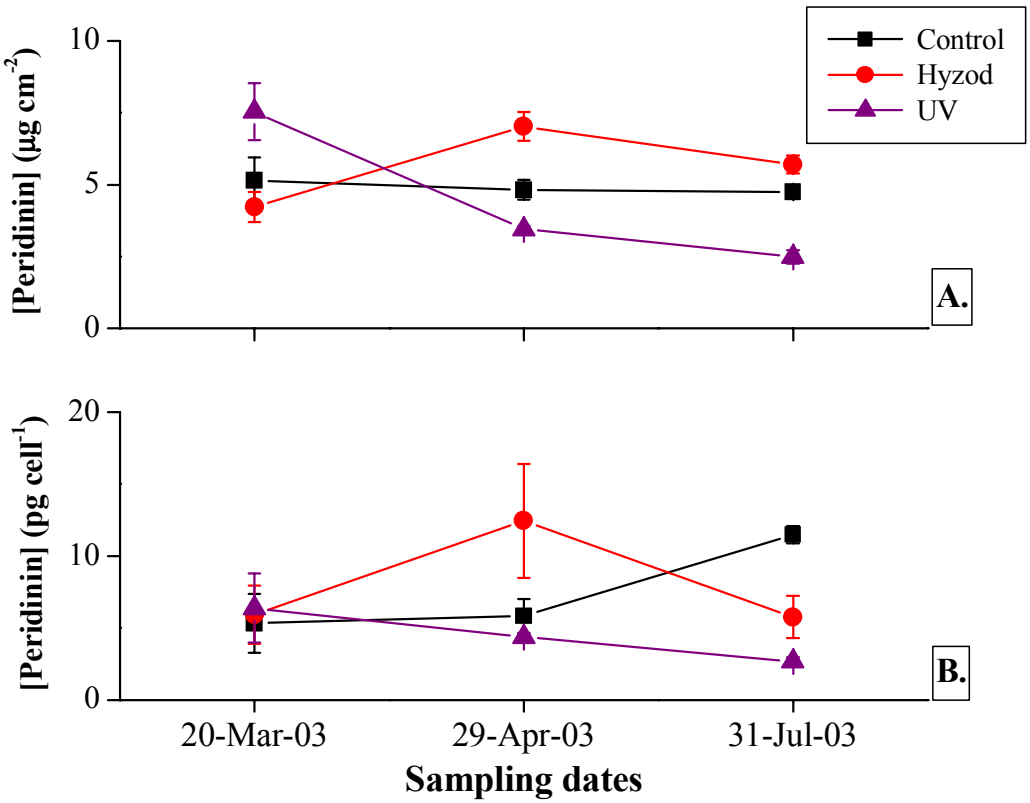


Figure 3.34. Peridinin concentration normalized to A) coral tissue area and B) zooxanthellae density in *P. furcata* during the UV-S Experiment. Vertical lines denote ±1SD.

**Zooxanthellae**

While colonies of *Acropora cervicornis* transplanted from the shelf edge to the back-reef of San Cristóbal Reef showed immediate signs of bleaching, no significant differences were found in zooxanthellae densities among treatments (Figure 3.35A; Fully Nested ANOVA, p=0.168). Similarly, no significant differences were found in the zooxanthellae mitotic index among treatments (Figure 3.35B Fully Nested ANOVA, p=0.173). Hence, bleaching in the studied colonies of *A. cervicornis* was produced by a decrease in zooxanthellae photosynthetic pigments rather than an expulsion of the host

symbionts. No differences in zooxanthellae density (Fully Nested ANOVA, p=0.981) or zooxanthellae mitotic index (Fully Nested ANOVA, p=0.194) were found between replicates among treatments in *A. cervicornis*. Control colonies at deeper areas varied during the experiment, while those colonies at shallower areas showed a constant decline in zooxanthellae densities as summer approached. Colonies transplanted from intermediate depths to deeper areas showed a slight decrease in zooxanthellae densities one month after the transplant. This was complemented by an increase in photosynthetic pigments (see above). Nonetheless, the increase in symbiont densities per coral tissue area suggests a recuperation of the sampled colonies.

The zooxanthellae densities of *P. furcata* colonies in the control and Hyzod® treatments varied during the UV-S Experiment with similar decreases after one month of exposure and a subsequent increase three months later (Figure 3.36A). While the zooxanthellae densities of the colonies of *P. furcata* exposed to enhanced UVR doses showed a decline with time of exposure, they were not significantly different from the zooxanthellae densities of colonies exposed to the other two treatments (Fully Nested ANOVA, p=0.068). Similarly, the mitotic index among treatments was not significantly different (see Figure 3.36B; Fully Nested ANOVA, p=0.371). Colonies at all treatments showed a decrease in mitotic index by the end of the UV-S Experiment. Hence, the bleaching event that occurred in the colonies of *P. furcata* was a result of a decrease in photosynthetic pigments in the coral host symbionts rather than a decrease in zooxanthellae densities. No differences in zooxanthellae density (Fully Nested ANOVA, p=0.641) or zooxanthellae mitotic index (Fully Nested ANOVA, p=0.123) were found between replicates among treatments in *P. furcata*.

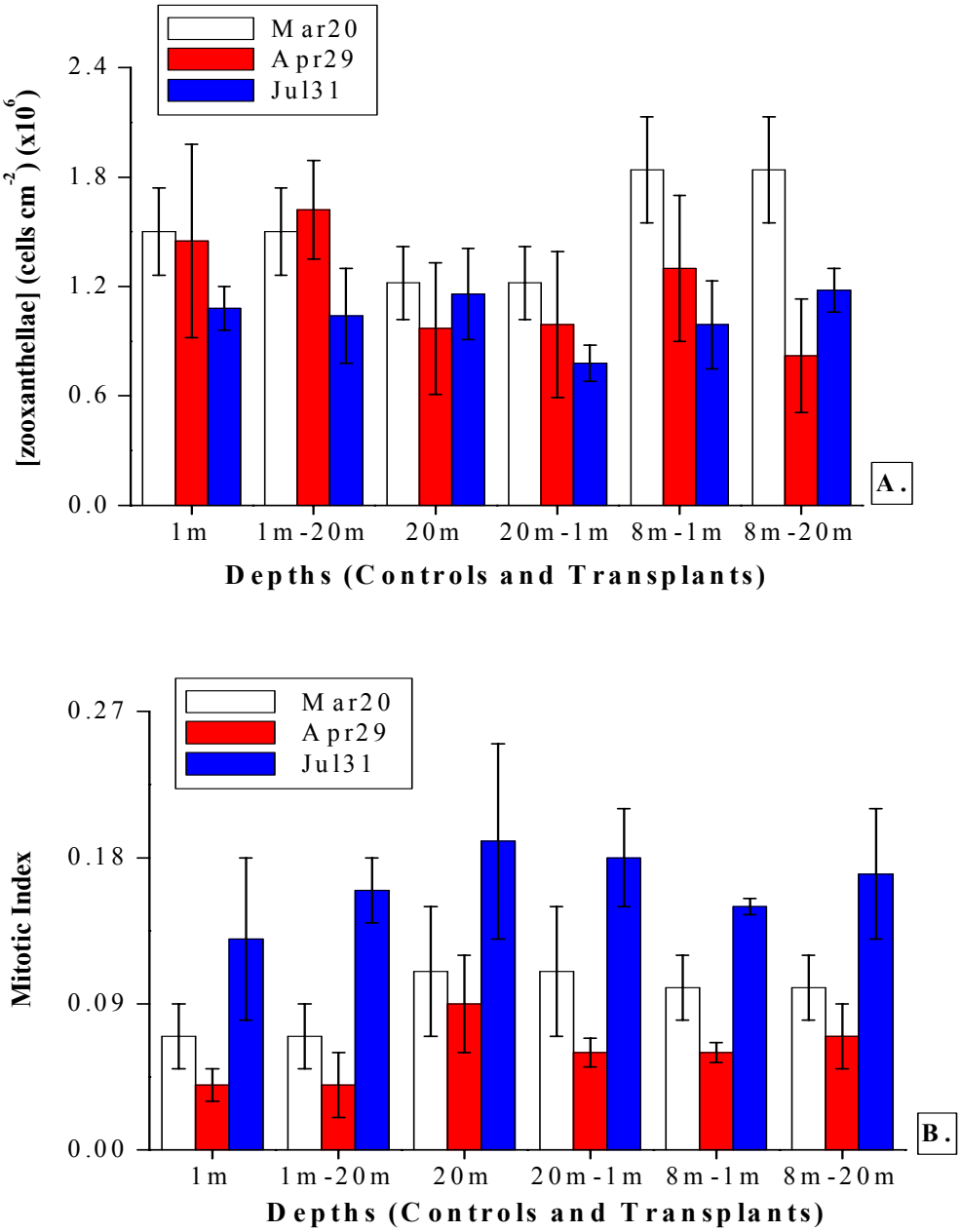


Figure 3.35. A) Zooxanthellae densities (normalized to coral tissue area) and B) Mitotic Index (MI) in *A. cervicornis* at the end of the UV-S Experiment. Legend: 1m = control colonies at the back-reef area of San Cristóbal Reef; 20m = control colonies at the Old Buoy shelf edge area; other columns refer to original vs. transplanted depth. MI is expressed as the amount of cells dividing/total amount of cells present in a given sample. Vertical lines denote ±1SD.

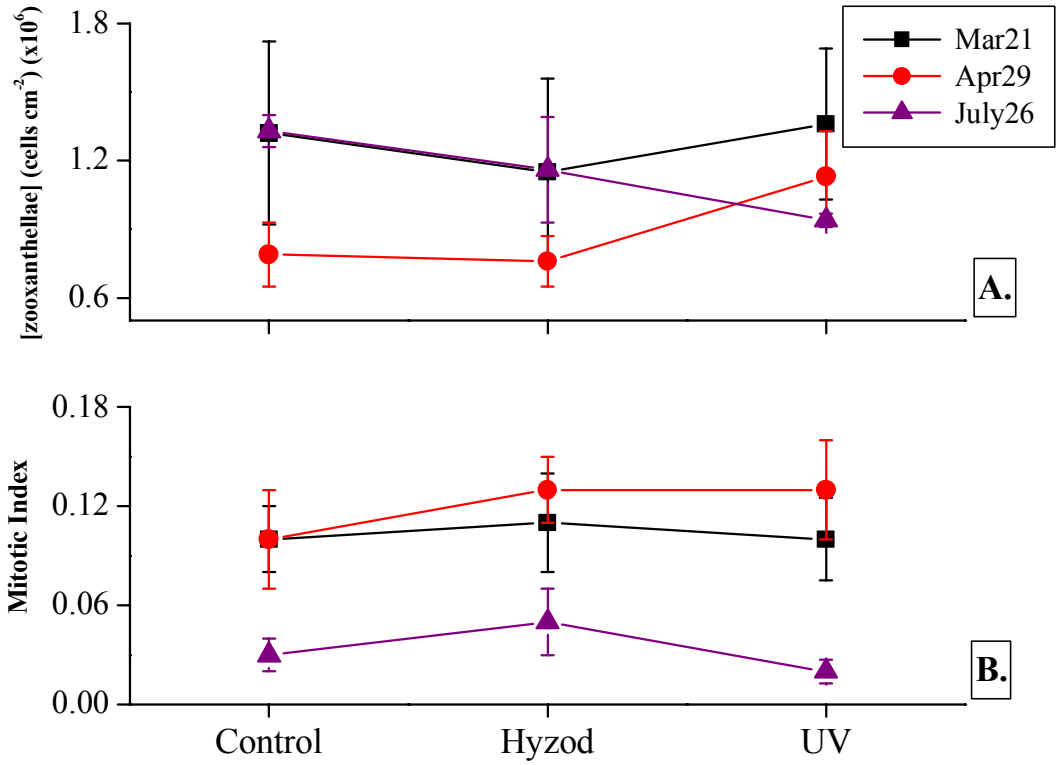


Figure 3.36. A) Zooxanthellae densities (normalized to coral tissue area) and B) Mitotic Index (MI) in *P. furcata* at the end of the UV-S Experiment. MI is expressed as the amount of cells dividing/total amount of cells present in a given sample. Vertical lines denote ±1SD.

# DISCUSSION

This experiment evaluated the effects of UVR on the photosynthetic and photoprotective pigments produced by two Caribbean ramose corals. The experimental setup consisted of two phases: 1) exclusion of UVR reaching the benthos at 1m depth at San Cristóbal Reef and, 2) increasing the daily doses that the corals receive at their normal growing depths.

## Mycosporine-Like Amino Acids (MAA's)

Two complications may arise in examining the prevalence of compounds in nature and their correlations with UV exposure: 1) the quenching of reactive oxygen species by carotenoids and phototaxis (this may provide the required UVR response that may negate the need for UV-screening compounds in some species); and 2) the compounds may have other physiological functions as well as UV screening (Cockell and Knowland, 1999). The significant 40-fold increase in total MAA's concentration in *Acropora cervicornis* after one month of exposure to enhanced UVR levels during the UV-S Experiment clearly indicates that, at least in this species, the major physiological function of these compounds is UVR protection.

While the penetration of ambient UVR through the coral tissue can be lower that of PAR (Shashar et al., 1997), the presence of MAA's can protect vital physiological functions from detrimental effects. MAA's such as mycosporine-glycine have moderate antioxidant activity and may provide some relief against photooxidative stress in the

hyperoxic tissues of algal-invertebrate symbiosis (Dunlap et al 1998). Proteins, lipids and DNA are the main targets of UV-B (Häder et al 2003).

The MAA's composition in algae (either free-living or symbiotic) appears to be species-specific since species from the same genus do not inevitably exhibit the same MAA composition (García-Pichel and Castenholz, 1993; García-Pichel et al., 1993; Karentz et al., 1991; Karsten et al., 1998; Rozema et al., 2002). Whereas the MAA's composition in the studied species during the present work was similar, a distinct preference in the production of the individual MAA's among species supports earlier findings of Kuffner et al. (1995) of an inverse relationship between MAA's concentration in four Hawaiian scleractinian corals (*Montipora verrucosa, M. patula, Pocillopora meandrina,* and *Porites compressa*) and depth of occurrence and dose of UVR although the MAA's varied in concentrations in the different species. For example, palythine had the highest concentration in *M. verrucosa* and *M. patula*, mycosporine-glycine was the highest in *P. meandrina*, and Asterina-330 was the highest in *P. compressa*. In a similar experiment, chlorophyll *a* and MAA's levels in colonies of *M. verrucosa* were not significantly different when exposed to full sunlight compared to those exposed to PAR only for two days (Lewis, 1995) indicating that short-term response to UVR may not be representative of conditions in nature that evolved over longer time scales. Nevertheless, Grottoli-Everett and Kuffner (1995) proved that bleaching (measured as changes in chlorophyll *a* concentration per zooxanthellae cell and colony area) can be unequally distributed within a colony exposed to elevated levels of PAR and UVR during nine days. Yet, the authors did not find any significant difference in zooxanthellae densities among the different UVR + PAR treatments. Only a significant increase in zooxanthellae

densities was found when corals were exposed to PAR only (Grottoli-Everett and Kuffner, 1995). Earlier, Lesser and Shick (1989) reported reduced levels of chlorophyll *a* in the sea anemone *Aiptasia pallida* in the presence of UVR.

The significant reduction in MAA's in both species under reduced UVR levels initially suggested a relationship between the production of these compounds and UVR similarly to earlier findings both in the Caribbean (Banaszak et al., 1998) and the Indo-Pacific (Dunlap and Chalker, 1986; Shick et al., 1995). However, only a handful of studies have analyzed the effects of enhanced doses of UVR by physically submitting corals to higher incident levels of natural UVR penetrating naturally clear reef waters. Gleason (1993) exposed colonies of *Porites astreoides* to enhanced UVR levels by transplanting colonies to shallower areas. A similar approach was followed in the present study with *A. cervicornis* colonies during the UV-S Experiment. A significant increase in the MAA's of these colonies was observed after the transplants despite a severe bleaching event in all colonies transplanted from the shelf edge at 20m depth to the back-reef area of San Cristóbal Reef at 1m depth. The increased concentration of all MAA's towards the experimental period positively correlated with increased UVR doses as daylength increased.

The colonies of *P. furcata* were exposed to artificially enhanced UVR levels in outdoor aquariums at Magueyes Island. Q-Panel UV-313nm fluorescent lamps supplied additional UVR at noon during the normal daily light cycle. Only one study (Shick et al., 1999) has followed a similar approached in the past. Shick et al. (1999) exposed colonies of the Indo-Pacific coral *Stylophora pistillata*, previously acclimated to the normal light received in indoor aquariums for four years, to increased UVR levels to study the

stimulation of the shikimate pathway for MAA's production. However, most of the increase in UVR levels during their experiment was in the UV-A region due to the use of UV-340nm lamps. The experiment of Shick et al. (1999) also lasted for 30 days. Yet, their results compare to those found here in the UV-S Experiment in terms of an increase in the accumulation of the MAA's despite colonies bleaching.

Earlier, Shick et al. (1995) found that in colonies transplanted from depths of 2, 10, 20, and 30m to 1m in the Great Barrier Reef, peak rates of photosynthesis in colonies from 2 and 10m were unaffected by the enhanced UVR, whereas photosynthesis was inhibited 30 and 38% in colonies transferred from 20 and 30m, respectively. They also found a 5-10-fold higher concentration of MAA's in the colonies from 2 and 10m compared to those of 20 and 30m in addition to a decline in the antioxidant enzyme activities in the zooxanthellae related to the decrease in potential for photooxidative stress with increasing depth.

Colonies of *Montipora verrucosa* acclimated to PAR and UVR showed higher levels of MAA's and lower zooxanthellae densities with no differences in chlorophyll a per host or symbiont cells (Kinzie, 1993). This author suggested that the fact that these corals respond to increases in UVR levels by increasing their MAA's concentrations indicates the active metabolism of these compounds whereas this expenditure of energy and resources were not incurred in low-UVR conditions. The results of the present study indicate that although UVR levels reaching deeper colonies (at 20m) are minimal, the coral symbionts still produce some of these compounds, especially those that absorb into the UVB region (i.e., mycosporine-glycine, 310nm peak maximum absorbance). As

showed in the Experimental Setup Section, UVR can penetrate to at least 20m depth in the clear insular shelf edge waters.

While the presence of MAA's in the mucus of the studied species was not addressed in this study, it is well-known that, Indo-Pacific scleractinian corals can transfer some of these UV-absorbing compounds to their mucous secretions as an additional protective sheet against UVR (Drollet et al., 1993; Teai et al., 1998). Drollet et al. (1993, 1997) reported positively correlated MAA's concentrations to solar UVR in the mucus of the Pacific coral *Fungia fungites* and *Fungia repanda*. Values for the concentration of MAA's reported here only represent those present within the host's zooxanthellae.

**Photosynthetic pigments and zooxanthellae densities**

Corals respond to stress events by changes in their growth rates (Torres, 2001), loss of zooxanthellae (Gleason and Wellington, 1993), aberrant fecundity (Szmant and Gassman, 1990), reduced planula larval survival (Baker, 1995), and changes in metabolism (Brown and Howard, 1985). Similarly, bleaching, either because of the loss the coral symbionts or their photosynthetic pigments, is frequently cited as a common response of scleractinian corals to stress events (see Glynn, 1993, 1996 for reviews on coral bleaching). Photosynthetic pigments can be bleached by UV-B and the structure of the light harvesting complexes is affected resulting in impaired photosynthesis (Häder et al 2003).

High irradiances in shallow-water may cause photoinhibition in reef coral zooxanthellae by means of reduced photosynthetic efficiency (e.g., quantum yield) or

capacity (e.g., photosynthetic rate) or both (Kirk, 1994; Winters et al., 2003). Winters et al. (2003) found a diurnal pattern in the effective quantum yield ($\Delta F/F'_m$) of *Stylophora pistillata* at 2m depth inversely related to incident downwelling PAR, whereas this pattern was not found in deeper colonies (11m depth) possibly due to a 3-fold decrease in PAR. Although Winters et al. (2003) did not measured incident UVR, this factor was not discarded as a possible source influencing their results. This problem aggravates in small-polyped coral species, such as *Acropora cervicornis* and *Porites furcata*, which are more dependent upon autotrophy for their nutrition than large-polyped species (i.e., *Montastraea cavernosa*) (Davies, 1991). Similar results have also been found in the red algae *Porphyra umbilicalis* (Gröniger et al., 1999). The authors found a slow but steady decline in the effective photosynthetic quantum yield from which there was no recovery, even when the decline did not exceeded 10%.

The most common cause of coral bleaching is temperature (Glynn, 1993; Brown et al., 1995; Fitt and Warner, 1995; Winter et al., 1998; Hoegh-Guldberg, 1999), while other causes such as sedimentation (Acevedo and Goenaga, 1986; Glynn, 1996) and increased intensities of UVR and PAR (Gleason and Wellington, 1993; Brown et al., 1995; Ambarsari et al., 1997) have been cited as other sources of bleaching. Symbioses with *Symbiodinium* species are exceptional in that they commonly live at 1-2$^{o}$C below the temperature which triggers a collapse of the symbiosis (i.e. bleaching) with negative consequences for the growth, reproduction and survival of the animal host. An explanatory framework for the causes of bleaching comprises three elements: the external factors or triggers of bleaching, (e.g. elevated temperature, UV, high irradiance, prolonged darkness, heavy metals, and pathogenic microorganisms), the symptoms,

including elimination of algal cells and loss of algal pigment, and the mechanisms, which define the response of the symbiosis to the triggers, resulting in the observed symptoms. Among corals, branching forms (e.g. *Acropora* and *Pocillopora* species) generally bleach more strongly than massive corals (Douglas, 2003). The determinants of this variation can be related to either the molecular physiology of *Symbiodinium* or the ecophysiology of the corals (Rowan and Powers, 1991; Rowan and Knowlton, 1995). Brown et al. (2000) showed that the bleaching susceptibility of the Pacific coral *Goniastrea aspera* can be predicted from its history of exposure to solar radiation, demonstrating how experience can shape coral bleaching patterns. In that case, exposure of the colonies to 34$^{o}$C resulted in significantly reduced algal density (bleaching) and caused an increase in the chlorophyll a content per algal cell similar to other findings in recent literature (Fitt et al, 1993; Jones, 1997b). However, high doses of UV can induce bleaching without increased temperature (Jokiel, 1980; Gleason & Wellington, 1993; present study).

Siebeck (1981, 1988) found that corals collected at depths of 18-20m were approximately twice as UV-sensitive as those from shallow waters. *Acropora cervicornis* colonies were severely bleached during the UV-S Experiment after one day of exposure when transplanted from 20m to 1m. Nonetheless, the bleaching occurred only on the sun-facing sides of the colonies. The bottom-facing side of the branches did not show any signs of bleaching at any time during the experiment. Since temperature changes were not significant between sites, these results present strong evidence on the importance of increases in UVR, and possibly PAR, in the short-term response of branching corals in the field.

While several authors have suggested that solar bleaching is a photochemical effect caused by increases in PAR levels rather than UVR (Brown et al., 1995, 2000a, b), the rapidly bleached branches of *A. cervicornis* just after one day of exposure to increased UVR and PAR irradiances strongly suggest that this can be a synergistic effect of both types of radiations. Similarly, the colonies of *Porites furcata* exposed to increased UVR levels in the outdoor aquariums showed bleaching signs during the experiment, but these were not as severe as those of *A. cervicornis*.

Symbiosis recombination with corals hosting highly temperature/irradiance resistant *Symbiodinium* clades, such as clade D (Baker, 2001, 2003; Baker et al., 2004), may enable some coral species to adapt to global warming and climate change (Buddemeier and Fautin, 1993; Buddemeier and Smith, 1999; Kinzie et al., 2001; Rowan, 2004). This "Adaptive Bleaching Hypothesis" may explain the resistance of *A. cervicornis* colonies after the severe bleaching that occurred to the upper sides of their branches after the transplant to shallower waters. Also, free-living *Symbiodinium* may be attracted specifically to host-lacking zooxanthellae (Fitt, 1985). Nevertheless, since temperature changes were not significant between the deep and shallow water sites, this factor can be disregarded as a possible influencing factor in the observed bleaching of this species. While there is a possibility of a re-colonization of the bleached branches by new highly resistant zooxanthellae from the field, the fact that the lower sides of the branches did not bleach during the whole experiment strongly suggests that an inter-polyps translocation of zooxanthellae cells may have occurred. Fitt et al. (2000) reported that during natural episodes of bleaching in *Montastraea annularis*, even severely bleached colonies of *M. annularis* retain at least 10% of their pre-bleaching population of

zooxanthellae. Also, there was an increase in the mitotic index by the end of the experiment which in turn may have contributed to the increase in chlorophyll *a* towards the end of the experiment. Moreover, it appears that within certain limits, UV-induced damage can be reversed by radiation of suitable wavelengths increasing the UV tolerance of the corals (Siebeck, 1988). The high survival rate of the severely bleached colonies transplanted from deep to shallow areas supports this hypothesis.

Bleaching can either be caused by an expulsion of the coral's symbionts or a decrease in their respective photosynthetic pigments, or both (Glynn, 1993, 1996; Hoegh-Guldberg, 1999). The evidence presented here indicates that bleaching [caused by enhanced UVR levels] in both studied species occurs mainly as a result of a decrease in the *Symbiodinium* photosynthetic pigments and less probably because of an expulsion of the zooxanthellae cells. Moreover, the variation in the zooxanthellae cells and mitotic indices suggest that these responses are probably an adaptation to other physical environmental factors such as changes in day length and UVR doses through the year. In 1987, 22% of the colonies observed at Cayo Enrique suffered partial or total bleaching; 69% only bleached in the upper tissues. No relationship was observed between depth and colonies bleached (Goenaga et al., 1989). The authors proposed that increased *in situ* solar radiation, related to doldrum conditions, and increased water temperature were the causing agents.

Fitt and Warner (1995) found an increase in chlorophyll *a* per zooxanthellae in the Caribbean reef-builder *Montastraea annularis* and suggested it was probably due to photoadaptation to the experimental light intensities, which were slightly lower than the

light intensities *in situ*. However, the very short term of their experiment (only 2 days) may influence any comparison with longer experiments such as this one.

Different species growing at the same depth may differ in their light-harvesting properties. However, for each species there may be an optimal depth (or irradiance level) for light-harvesting. To meet carbon-fixation requirements in deeper water, or in the shade, zooxanthellae have developed adaptations for light harvesting such as changes in enzyme activities and increases in the concentration of chlorophyll *a* as well as accessory pigments such as chlorophyll $c_2$, and peridinin (Gil-Turnes and Corredor, 1981; Muscatine, 1990). Both species, *A. cervicornis* and *P. furcata*, showed increases, yet not significant, in zooxanthellae densities when exposed to decreased UVR and PAR levels throughout the UV-E Experiment. However, exposure of both species to increased levels of UVR and PAR did not significantly affect the zooxanthellae densities during the UV-S Experiment. This suggests that even though these species harbor different clades of *Symbiodinium* (Baker and Rowan, 1997; Rowan, 1998; LaJeunesse et al., 2003), their response to stressful conditions is similar in terms of reducing their photosynthetic pigments concentrations while maintaining their population densities *in hospite* at close-to-normal levels by increasing their rate of cell division (see Figure 3.46). The reduction in photosynthetic pigments concentration suggests a possible defensive mechanism itself to reduce the chance of photodamage to the light-harvesting systems, especially PSII (see Warner et al., 1999, 2002), in the zooxanthellae cells.

Data from Discovery Bay, Jamaica from Wyman et al. (1987) showed values of chlorophyll *a* for *A. cervicornis* of 1.3 (1m), 2.94 (10m) and 2.52 (30m) Chl *a*/cell (pg chl *a* $cell^{-1}$) and Chl *a*/area (μg chl *a* $cm^{-2}$) of 2.74 (1m), 6.01 (10m) and 2.96 (30m); also for

*P. astreoides* of 2.93 (1m), 3.78 (10m) and 3.98 (30m) Chl *a*/cell, and 15.64 (1m), 17.46 (10m) and 9.54 (30m) Chl *a*/area suggesting a maximum light-harvesting capability at approximately 10m for both species. The results obtained during the UV-S Experiment differ from those of Wyman et al. (1987); the concentration of chlorophyll *a* either per host tissue area or per zooxanthellae cell increased with depth in *A. cervicornis*. Similarly, during the UV-E Experiment both species increased their respective photosynthetic pigments with decreasing UVR and PAR either under the Hyzod® or Saran® treatments.

Dustan (1979, 1982) found that zooxanthellae taken from corals growing at shallow depths absorb less light energy than cells taken from corals growing in deep waters which show greater whole-cell absorption, resulting from the accumulation of pigments offsetting the decrease in available light with increasing depth. In fact, in some corals, the density of zooxanthellae may increase with decreasing light, which also results in overall increased light absorption (Falkowski et al., 1990). Pigment concentration (chlorophylls and carotenoids) relative to zooxanthellae cells occurs in an opposite way to that of the photosynthetic capacity of *A. cervicornis* (Chalker and Taylor, 1978).

The role of carotenoids in UV screening is still somewhat controversial, yet it is known that in many cases they are UV-inducible. Their ability to transfer energy means they are important in photosynthetic light reactions and they may have a primary role in photosynthesis. Their role in quenching reactive oxygen species could have evolved during the increase in atmospheric partial pressures in the early Proterozoic (Krinsky, 1971) or perhaps earlier in phototrophic organisms producing oxygen in their micro-

environments. Thus, they may have taken on a photoprotective role as an additional function in the earliest oxygenic photosynthesizers.

When an alga is exposed to high irradiances, diatoxanthin (DT) is formed at the expense of diadinoxanthin (DD) (Roberfroid and Calderon, 1995). On the return of the alga to lower irradiance, diadinoxanthin is produced, albeit at a slower rate than the initial de-epoxidation (Frank et al., 1996). Only about 60% of the chloroplast diadinoxanthin appears to be available for de-epoxidation. Similarly, changes in the diadinoxanthin/diatoxanthin pool size can be correlated with irradiance levels during algal growth. The operation of the xanthophyll cycle and the subsequent dissipation of excess excitation energy is an important contribution to the protection of the photosynthetic apparatus from light-induced damage. The enzymatic de-epoxidation/epoxidation reactions of the xanthophyll cycle may be acting as regulator of light flow in the pigment protein complexes. Under conditions of low light, the carotenoid could act as a light-harvesting pigment transferring energy to the chlorophyll. Under excess light conditions, the system could transfer the energy of the chlorophyll back to the carotenoid (Frank et al., 1996)

The colonies of *A. cervicornis* showed signs of recuperation (i.e., increased chlorophyll *a* and diadinoxanthin concentration per coral tissue area) at the end of the experiment (see Table 3.7). While seasonal alterations in protein and pigment content (Fagoonee et al., 1999) may significantly influence patterns of dynamic photoinhibition originating from the light-harvesting antennae and this can be especially important in corals residing in shallow habitats where xanthophyll cycling contributes to photoprotection (Warner et al., 2002), the variation and further decrease in xanthophyll

ratio (DT/DD+DT) in both species at the end of the UV-S Experiment indicates another adaptation of the colonies to increased irradiance levels.

The identification of pigments such as zeaxanthin and fucoxanthin in some samples might be indicative of the presence of other organisms, not necessarily symbionts, in the corals. For instance, these are signature pigments of cyanobacteria and diatoms, respectively (Jeffrey et al., 1997; 1999). The variation in accessory pigments such as β, β-carotene, the diadinochromes I and II, P-457, and P-468 presents additional evidence on the presence of different zooxanthellae clades in both species. The relative importance of these pigments on each species and how their concentrations correlate to their colorations can be calculated by analyzing the second and fourth derivaties of their respective absorption signatures (JL Torres, unpublished data).

There are significant synergistic effects of solar UVR with oxidative stress in marine organisms. The reactions leading to reactive oxygen species (ROS) such as superoxide radicals ($O_2^-$), hydrogen peroxide ($H_2O_2$) and hydroxyl radicals ($HO^-$) are know to occur normally in metabolic processes in the cytosol, mitochondria, and chloroplasts (Halliwell & Gutteridge, 1999), but concentrations of these radicals increase under stressful conditions (Fridovich, 1986). ROS also have multiple adverse effects on marine organisms including damage to DNA/RNA, inhibition of photosynthesis, apoptosis, and bleaching of zooxanthellate corals (Dykens et al., 1992; Shick et al 1995; Lesser, 1996, 1997). Defenses against ROS include various non-enzymatic antioxidants and quenchers (e.g., ascorbate, carotenoids) (Fridovich, 1986; Peak and Peak, 1990).

Loss of zooxanthellae can be used for assessing stress in corals, yet inherent differences in the densities of zooxanthellae between coral colonies are likely to affect

the outcome of experiments using the bleaching response and other biochemical or physiological stress responses (Jones, 1997a). Published zooxanthellae densities for the studied species are: *P. furcata* $4.26x10^6$ cells/$cm^2$ (Meyer and Schultz, 1985); *A. cervicornis* $2.11x10^6$ cells/$cm^2$ (Wyman et al., 1987). Both *A. cervicornis* and *P. furcata* showed similar densities during the present study with a tendency of increasing their populations as the light regime decreases. Stimson et al. (2002) presents a table showing also an apparent decrease in zooxanthellae densities in *Montastraea* with increasing depth, yet this might be due to misidentified species within the complex since they were all identified at all depths as *M. annularis*. Also, species-specific patterns of depth-dependent photosynthesis in corals seem more likely to occur than a general response. For instance, the ratio of chlorophyll *a*:$c_2$ in the coral *Acropora microphthalma* decreased with increasing depth. This represents compensation in the photosynthetic apparatus to lower availability of PAR, which at 30m declines to 8.2% of surface irradiance at the study site (Shick et al., 1995).

Zooxanthellae produced from algal division in excess of those released may either be taken up by newly formed endodermal cells or used to balance the loss of pycnotic zooxanthellae. Alternatively, zooxanthellae may be released as a result of necrosis, apoptosis (programmed cell death), or adhesion dysfunction of algal bearing host endodermal cells (Jones, 1997b). The coral colonies studied by Jones gradually recovered their coloration suggesting that bleaching, in this case, was a sublethal response. He found no significant differences in mitotic index among treatments. It averaged less than 1% at all treatments. He argued that using the mitotic index may introduce errors in the interpretations due to: 1) corals in the process of losing zooxanthellae will have higher

ratios of extracellular to intracellular zooxanthellae which may increase the MI of the algal population; 2) increases and decreases can occur during, and/or after an external stress, producing a generally obscure picture of the stress response.

The mitotic index of zooxanthellae in non-*Porites* corals is inversely correlated to their density (Stimson et al., 2002). *Porites* densities differ in having substantially higher mitotic indices than the other genera for a given density of zooxanthellae. Mitotic indices did not differ significantly between the present coral species studied, with high and low bleaching-induced mortality even though species with high bleaching-induced mortality tended to have high densities of zooxanthellae. Yet, the overall relationship seems to be a negative one.

The hypothesis that branching species or species with high growth rates and metabolic rates (Hoegh-Guldberg and Smith, 1989) are more susceptible to bleaching cannot be applied to all species. The present study shows that, at least in the Caribbean, Acroporid species might be more susceptible to bleaching than Poritids. Even with an increase in the actual levels of UVR, especially UV-B, the bleaching of the *P. furcata* colonies located in the aquariums under the UV lamps was more conservative than in *A. cervicornis* colonies transplanted to shallow waters in the field. Therefore, it is concluded that while temperature may vary constantly in Caribbean reef waters, it is demonstrated that the incidence of high PAR and more importantly UVR doses can be a major cause of coral bleaching in the zone. Furthermore, these results provide insights as to how valid are experiments performed in more controlled environments (Carlson, 1999), and if these can be used as representatives of possible changes in the environmental parameters that might occur in the real world.

# GENERAL DISCUSSION AND CONCLUSIONS

Predictions of the response of entire ecosystems to elevated UVB cannot be made on single trophic-level assessments. Bothwell et al. (1994) found that even with a 50% reduction in overall solar intensity, the presence of UVR exerted a negative effect on the number of organisms studied at the ecosystem level. Because ozone is a highly selective absorber of UVB, ozone decreases will not only increase UVB, but will also elevate the ratio of UVB to longer wavelengths, depriving animals of their natural environmental cue to the presence of harmful levels of UVB.

The diversity in physiological functions is one of the complications in studying UV-screening compounds and determining the true ecological importance of their UV-screening role. As well as providing protection against ambient UV radiation, species with effective screening may also be at an advantage during natural ozone depletion events.

Brown et al. (2000) found that an exposure of *Goniastrea aspera* colonies to 34$^{o}$C resulted in significantly reduced algal density (bleaching) and caused an increase in the chlorophyll *a* content per algal cell similar to other findings in recent literature (Fitt et al., 1993; Jones, 1997). However, high doses of UV can induce bleaching without increased temperature (Jokiel, 1980; Gleason & Wellington, 1993). The present results fully support the latter ideas. In general, a significant bleaching event, primarily indicated by a reduction in the photosynthetic algal pigments, was caused in both species when exposed to artificially or naturally enhanced UVR doses under similar temperature regimes.

The results of this study suggest that experiments based on exposing the corals to reduced UVR levels and extrapolating possible effects of enhanced UVR situations may not be realistic. This work presents evidence that an enhanced UVR scenario can be simulated with great success either in the field (by transplanting the organisms to different depths) or under more controlled situations in the laboratory (by supplementing UVR artificially with UV fluorescent lamps).

The results on the variability in the relative contribution of individual MAA's and photosynthetic pigments detected in the extracts of both studied species support earlier findings that different *Symbiodinium* clades are present among the two species (Baker and Rowan, 1997; Baker et al., 1997; LaJeunesse 2002; LaJeunesse et al., 2003). More studies correlating the presence of different MAA's in different coral species and their respective *Symbiodinium* clades should be performed in order to obtain more information on the adaptation of these symbionts to different environmental factors.

It is also concluded that even slight increases in UVR dose can produce devastating effects on important reef coral physiological functions such as skeletal formation and growth as well as reproduction. Corals exposed to enhanced UVR levels, either naturally or artificially reduced, significantly their linear extension rates between 25 and 66%. These reductions were found to be species dependent with *Acropora cervicornis* being more susceptible to changes in UVR than *Porites furcata*. Furthermore, both species produced more fragile skeletons reflected in a reduction in their skeletal densities.

The studied species also differ in their sexual reproductive output to enhanced UVR. Colonies of *A. cervicornis* showed a 100% reduction in number of gonads per

polyp after being transplanted to shallower waters suggesting reabsorption of at least the eggs. This response is possibly a strategy used by reef corals to translocate these resources for usage in other physiological aspects such as production of UV-absorbing compounds by their zooxanthellae in order to ensure colony survival after a sever bleaching event. On the other hand, while *P. furcata* colonies exposed to artificially enhanced UVR doses showed a reduction in the number of eggs and spermaries after several weeks of exposure, the colonies were able to recuperate and continued to produce gonads throughout the experiment.

It has been suggested (Smith and Buddemeier, 1992), and recently demonstrated (Gattuso et al., 1999; Kleypas et al., 1999), that an increase in the $CO_2$ partial pressure ($pCO_2$) has a negative effect on coral and reef community calcification as a result of a decrease in the aragonite saturation state ($\Omega_{arag}$). The increase in atmospheric $pCO_2$ forecast for the next century led these authors to predict a significant decrease of marine calcification. Furthermore, Leclerq et al. (2002) found a reduction in the rate of net coral community calcification as a function of increasing $pCO_2$ and decreasing aragonite saturation state re-emphasizing the predictions that reef calcification is likely to decrease during the next century.

A decrease of $\Omega_{arag}$ from its pre-industrial value (4.9) to its predicted value for 2100 (3.0) has been predicted (Leclerq et al., 2002). Hence, a reduction in the calcification rates of scleractinian corals and red calcareous algae by 11 and 32%, respectively is expected. The present results support this hypothesis and further indicate the importance of UVR in augmenting these predictions.

Considering the current status of Caribbean acroporids (*Acropora* Review Team, 2005) the results present an alarming scenario for the upcoming years. Further studies regarding the genetic composition and diversity of this genus in the Caribbean are needed to fully evaluate the effects of UVR, decreased aragonite state, sedimentation, temperature, and other influencing factors on the physiological responses of these important Caribbean reef-builders. Also, detailed studies on the relative importance of sexual versus asexual reproduction at the genus and species level are needed in order to design proper management efforts to ensure their short and long term survival.

## REFERENCES


Acevedo R and C Goenaga. 1986. Note on coral bleaching after a chronic flooding in southwestern Puerto Rico. Carib. J. Sci. 22(3-4): 225.

Ackleson SG. 2003. Light in shallow waters: a brief research review. Limnol. Oceanogr. 48(1, part 2): 323-328.

*Acropora* Review Team. 2005. Atlantic *Acropora* status review document. Report to National Marine Fisheries Service (NOAA). Southeast Regional Office. 152pp + App.

Adams NL and JM Shick. 1996. Mycosporine-like amino acids provide protection against ultraviolet radiation in eggs of the sea urchin *Strongylocentrotus droebachiensis*. Photochem. Photobiol. 64(1): 149-158.

Adams NL and JM Shick. 2001. Mycosporine-like amino acids prevent UVB-induced abnormalities during early development of the green sea urchin *Strongylocentrotus droebachiensis*. Mar. Biol. 138: 267-280.

Adams NL, JM Shick, and WC Dunlap. 2001. Selective accumulation of mycosporine-like amino acids in ovaries of the green sea urchin *Strongylocentrotus droebachiensis* is not affected by ultraviolet radiation. Mar. Biol. 138: 281-294.

Ambarsari I, BE Brown, RG Barlow, G Britton, and D Cummings. 1997. Fluctuations in algal chlorophyll and carotenoid pigments during solar bleaching in the coral *Goniastrea aspera* at Phuket, Thailand. Mar. Ecol. Prog. Ser. 159: 303-307.

Bradford MM. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochem. 72: 248-254.

Bak RPM and SR Criens. 1981. Survival after fragmentation of colonies of *Madracis mirabilis, Acropora palmata* and *Acropora cervicornis* (Scleractinia) and the subsequent impact of a coral disease. Proc. 4th Coral Reef Symp. Manila. 2: 221-227.

Baker AC. 1995. Solar UV-A inhibition of planula larvae in the reef-building coral *Pocillopora damicornis*. In: Gulko D and PL Jokiel (eds) Ultraviolet Radiation and Coral Reefs. HIMB Tech. Report #41. UNIHI-Sea Grant-CR-95-03. pp. 149-163.

Baker AC. 2001. Reef corals bleach to survive change. Nature. 411: 765-766.

Baker AC. 2003. Flexibility and specificity in coral-algal symbiosis: diversity, ecology, and biogeography of *Symbiodinium*. Annu. Rev. Ecol. Evol. Syst. 34: 661-689.

Baker AC and R Rowan. 1997. Diversity of symbiotic dinoflagellates (zooxanthellae) in scleractinian corals of the Caribbean and Eastern Pacific. Proc. 8th Int. Coral Reef Symp. Panama. 2: 1301-1306.

Baker AC, R Rowan, and N Knowlton. 1997. Symbiosis ecology of two Caribbean acroporid corals. Proc. 8th Int. Coral Reef Symp. Panama. 2: 1295-1300.

Baker AC, CJ Starger, TR McClanahan, and PW Glynn. 2004. Corals' adaptive response to climate change. Nature. 430: 741.

Baker K, RC Smith, and AFS Green. 1980. Middle ultraviolet radiation reaching the ocean surface. Photochem. Photobiol. 32: 367-374.

Balch WM and FT Haxo. 1984. Spectral properties of *Noctiluca militaris* Suriray, a heterotrophic dinoflagellate. J. Plankton Res. 6: 515-525.

Banaszak AT and RK Trench. 1995a. Effects of ultraviolet (UV) radiation on marine microalgal-invertebrate symbioses. I. Response of the algal symbionts in culture and *in hospite*. J. Exp. Mar. Biol. Ecol. 194: 213-222.

Banaszak AT and RK Trench. 1995b. Effects of ultraviolet (UV) radiation on marine microalgal-invertebrate symbioses. II. The synthesis of mycosporine-like amino acids in response to exposure to UV in *Anthopleura elegantissima* and *Cassiopeia xamachana*. J. Exp. Mar. Biol. Ecol. 194: 233-250.

Banaszak AT, MP Lesser, IB Kuffner, and M Ondrusek. 1998. Relationship between ultraviolet (UV) radiation and mycosporine-like amino acids (MAAS) in marine organisms. Bull. Mar. Sci. 63(3): 617-628.

Banaszak AT, TC LaJeunesse, and RK Trench. 2000. The synthesis of mycosporine-like amino acids (MAAs) by cultured, symbiotic dinoflagellates. J. Exp. Mar. Biol. Ecol. 249: 219-233.

Barnes, DJ. 1973. Growth in colonial scleractinians. Bull. Mar. Sci. 23(2): 280-298.

Barnes DJ and BE Chalker. 1990. Calcification and photosynthesis in reef-building corals and algae. In: Dubinsky Z (ed.) Ecosystems of the World: Coral Reefs. Elsevier. Amsterdam, The Netherlands. pp. 109-131.

Battey JF and JW Porter. 1988. Photoadaptation as a whole organism response in *Montastraea annularis*. Proc. 6th Int. Coral Reef Symp. Australia. 3: 79-85.

Bentley R. 1990. The Shikimate Pathway – A metabolic tree with many branches. In:(editors) Critical Reviews in Biochemistry and Molecular Biology. CRC Press. Florida, USA. 25(5): 307-384.

Bidigare RR. 1991. Analysis of algal chlorophylls and carotenoids. In: Hurd DC and DW Spencer (eds.) Marine particles: analysis and characterization. Geophysical Monograph 63. American Geophysical Union. pp.119-123.

Bijlsma L, CN Ehler, RJT Klein, SM Kulshrestha, RF McLean, N Mimura, et al. 1995. Coastal zones and small islands. In: Watson RT, MC Zinyowera, and RH Moss (eds.) Climate change 1995 – Impacts, adaptations and mitigations of climate change: Scientific-Technical Analyses: the Second Assessment Report of the Inter-Governmental Panel on Climate Change. Cambridge Univ. Press. New York. pp. 6-12.

Bothwell AM. 1981. Fragmentation, a means of asexual reproduction and dispersal in the coral genus *Acropora* (Scleractinia: Astrocoeniida: Acroporidae) – a preliminary report. Proc. 4th Int. Coral Reef Symp. Manila. 2: 137-144.

Bothwell ML, DMJ Sherbot, and CM Pollock. 1994. Ecosystem response to solar ultraviolet-B radiation: influence of trophic-level interactions. Science. 265: 97-100.

Bradford MM. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the Principle of Protein-Dye Binding. Anal. Biochem. 72: 248-254.

Brandt J. 1883. Über die morphologische und physiologische Bedeutung des Chlorophylls bei Tieren. Zool. Stn. Neapol. 4: 191-302.

Brown BE. 1988. Assessing the environmental impacts on coral reefs. Proc. 6th Int. Coral Reef Symp. Australia. 1: 71-87.

Brown BE and LS Howard. 1985. Assessing the effects of "stress" on reef corals. Adv. Mar. Biol. 22: 1-63.

Brown BE, MDA Le Tissier and JC Bythell. 1995. Mechanisms of bleaching deduced from histological studies of reef corals sampled during a natural bleaching event. Mar. Biol. 122: 655-663.

Brown BE, I Ambarsari, ME Warner, WK Fitt, RP Dunne, SW Gibb, and DG Cummings. 1999. Diurnal changes in photochemical efficiency and xanthophyll concentrations in shallow water reef corals: evidence for photoinhibition and photoprotection. Coral Reefs. 18: 99-105.

Brown BE, RP Dunne, MS Goodson, and AE Douglas. 2000a. Bleaching patterns in reef corals. Nature. 404: 142-143.

Brown BE, RP Dunne, ME Warner, I Ambarsari, WK Fitt, SW Gibb, and DG Cummings. 2000b. Damage and recovery of Photosystem II during a manipulative field experiment on solar bleaching in the coral *Goniastrea aspera*. Mar. Ecol. Prog. Ser. 195: 117-124.

Brown J, A Colling, D Park, J Phillips, D Rothery, and J Wright. 1989. Waves, tides and shallow-water processes. The Open University. England. 187pp.

Buddemeier RW and DG Fautin. 1993. Coral bleaching as an adaptive mechanism. Bioscience. 43: 320-326.

Buddemeier RW and SV Smith. 1999. Coral adaptation and acclimatization: a most ingenious paradox. Amer. Zool. 39: 1-9.

Calkins J. 1982. The role of solar ultraviolet radiation in marine ecosystems. Plenum Press. New York. 724 pp.

Calkins J and T Thordardottir. 1980. The ecological significance of solar UV radiation on aquatic ecosystems. Nature. 283: 563-566.

Carlson BA. 1999. Organism responses to rapid change: what aquaria tell us about nature. Amer. Zool. 39: 44-55.

Carreto JI, MO Carignan, G Daleo, and SG De Marco. 1990. Occurrence of mycosporine-like amino acids in the red-tide dinoflagellate *Alexandrium excavatum* – UV-photoprotective compounds? J. Plankton Res. 12: 909-921.

Carreto JI, MO Carignan, and NG Montoya. 2005. A high-resolution reverse-phase liquid chromatography method for the analysis of mycosporine-like amino acids (MAAs) in marine organisms. Mar. Biol. 146: 237-252.

Carroll AK and JM Shick. 1996. Dietary accumulation of UV-absorbing mycosporine-like amino acids (MAAs) by the green sea urchin (*Strongylocentrotus droebachiensis*). Mar. Biol. 124: 561-569.

Chalker BE. 1977. Daily variation in the calcification capacity of *Acropora cervicornis*. Proc. 3rd Int. Coral Reef Symp. 2: 417-423.

Chalker BE and WC Dunlap. 1981. Extraction and quantitation of endosymbiotic algal pigments from reef-building corals. Proc. 4th Int. Coral Reef Symp. 2: 45-50.

Chalker BE and DL Taylor. 1975. Light-enhanced calcification, and the role of oxidative phosphorylation in calcification of the coral *Acropora cervicornis*. Proc. R. Soc. London. B. 190: 323-331.

Chalker BE and DL Taylor. 1978. Rhythmic variations in calcification and photosynthesis associated with the coral *Acropora cervicornis* (Lamarck). Proc. R. Soc. London. B. 201: 179-189.

Chapman S. 1930. A theory of upper atmospheric ozone. Mem. Roy. Meteorol. Soc. 3: 103-125.

Chioccara F, A Della Galla, M de Ross, E Novellino, and G Prota. 1980. Mycosporine aminoacids and related compounds from the eggs of fishes. Bull. Soc. Chim. Belgique. 89: 1101-1106.

Chornesky EA and EC Peters. 1987. Sexual reproduction and colony growth in the scleractinian coral *Porites astreoides*. Biol. Bull. 172: 161-177.

Cockell CS and J Knowland. 1999. Ultraviolet radiation screening compounds. Biol. Rev. 74: 311-345.

Coles SL and PL Jokiel. 1978. Synergistic effects of temperature, salinity and light on the hermatypic coral *Montipora verrucosa* (Lamarck). Mar. Biol. 49: 187-195.

Corredor JE, AW Bruckner, FZ Muszynski, RA Armstrong, R García, and JM Morell. 2000. UV-absorbing compounds in three species of Caribbean zooxanthellate corals: depth distribution and spectral response. Bull. Mar. Sci. 67(2): 821-830.

Cullen JJ and MP Lesser. 1991. Inhibition of photosynthesis by ultraviolet radiation as a function of dose and dosage rate: results for a marine diatom. Mar. Biol. 111: 183-190.

Davies PS. 1991. Effect of daylight variations on the energy budgets of shallow-water corals. Mar. Biol. 108: 137-144.

D'Croz L, JL Maté, and JE Oke. 2001. Responses to elevated sea water temperature and UV radiation in the coral *Porites lobata* from upwelling and non-upwelling environments on the Pacific coast of Panama. Bull. Mar. Sci. 69(1): 203-214.

Demers S, S Roy, R Gagnon and C Vignault. 1991. Rapid light-induced changes in cell fluorescence and in the xanthophyll-cycle pigments of *Alexandrium excavatum* (Dinophyceae) and *Thalassiosira pseudonana* (Bacillariophyceae): a photo-protection mechanism. Mar. Ecol. Prog. Ser. 76: 185-193.

Detrés Y. 2001. Effects of ultraviolet radiation on the tropical marine macrophyte *Thalassia testudinum* (König). Ph.D. Thesis. Univ. of PR. 138 pp.

Detrés Y, RA Armstrong, and XM Connelly. 2001. Ultraviolet-induced responses in two species of climax tropical marine macrophytes. J. Photochem. Photobiol. B. 62: 55-66.

Dey DB and DM Damkaer. 1990. Effects of spectral irradiance on the early development of Chinook salmon. Prog. Fish-Cult. 52(3): 141-154.

Dieppa-Ayala A. 1996. Bio-optical variability in tropical oceanic waters. MS Thesis. Univ. of PR. 90pp.

Diffey BL. 1991. Solar ultraviolet radiation effects on biological systems. Rev. Phys. Med. Biol. 36(3): 299-328.

Dionisio-Sese ML, M Ishikura, T Maruyama, and S Miyachi. 1997. UV-absorbing substances in the tunic of a colonial ascidian protect its symbiont, *Prochloron* sp., from damage by UV-B radiation. Mar. Biol. 128: 455-461.

Douglas AE. 2003. Coral bleaching – how and why? Mar. Poll. Bull. 46: 385-392.

Dove SG, O Hoegh-Guldberg and S Ranganathan. 2001. Major colour patterns of reef-building corals are due to a family of GFP-like proteins. Coral Reefs. 19: 197-204.

Drollet JH, P Glaziou, and PMV Martin. 1993. A study of mucus from the solitary coral *Fungia fungites* (Scleractinia: Fungiidae) in relation to photobiological UV adaptation. Mar. Biol. 115: 263-266.

Drollet JH, T Teai, M Faucon, and PMV Martin. 1997. Field study of compensatory changes in UV-absorbing compounds in the mucus of the solitary coral *Fungia repanda* (Scleractinia: Fungiidae) in relation to solar UV radiation, sea-water temperature, and other coincident physico-chemical parameters. Mar. Freshwater Res. 48: 329-333.

Dunlap WC and BE Chalker. 1986. Identification and quantification of near-UV absorbing compounds (S-320) in a hermatypic scleractinian. Coral Reefs. 5: 155-159.

Dunlap WC, BE Chalker, and JK Oliver. 1986. Bathymetric adaptations of reef corals at Davies Reef, Great Barrier Reef, Australia. III. UV-B absorbing compounds. J. Exp. Mar. Biol. Ecol. 104: 239-248.

Dunlap WC, BE Chalker, and WM Bandaranayake. 1988. Ultraviolet light absorbing agents derived from tropical marine organisms of the Great Barrier Reef, Australia. Proc. 6$^{th}$ Int. Coral Reef Symp., Australia. 3: 89-93.

Dunlap WC, Masaki K, Yamamoto Y, Larsen RM, and Karube I. 1998. A novel antioxidant derived from seaweed. In: Le Gal Y and H Halvorson (eds.) Recent Developments in Marine Biotechnology. Plenum Press. pp 33-35.

Dustan P. 1979. Distribution of zooxanthellae and photosynthetic chloroplast pigments of the reef-building coral *Montastraea annularis*, Ellis and Solander, in relation to depth on a West Indian reef. Bull. Mar. Sci. 29: 79-95.

Dustan P. 1982. Depth-dependent photoadaptation by zooxanthellae of the reef coral *Montastraea annularis*. Mar. Biol. 68: 253-264.

Dykens JA and JM Shick. 1984. Photobiology of the symbiotic sea anemone, *Anthopleura elegantissima*: defenses against photodynamic effects, and seasonal photoacclimatization. Biol. Bull. 167: 683-697.

Dykens J, JM Shick, C Benoit, GR Buettner, and GW Winston. 1992. Oxygen radical production in the sea anemone *Anthopleura elegantissima* and its endosymbiotic algae. J. Exp. Biol. 168: 219-241.

Edmunds PJ and PS Davies. 1989. An energy budget for *Porites porites* (Scleractinia), growing in a stressed environment. Coral Reefs. 8: 37-43.

Fadlallah YH. 1983. Sexual reproduction, development and larval biology in scleractinian corals. Coral Reefs. 2: 129-150.

Fagoonee I, HB Wilson, MP Hassell, and JR Turner. 1999. The dynamics of zooxanthellae populations: a long-term study in the field. Science. 283: 843-845.

Falkowski PG, PL Jokiel, and RA Kinzie III. 1990. Irradiance and corals. In: Dubinsky Z (ed.) Ecosystems of the World: Coral Reefs. Elsevier. Amsterdam, The Netherlands. pp. 89-107.

Farman JC, BG Gardiner, and JK Oliver. 1986. Large losses of total ozone in Antarctica reveal seasonal Clox/NOx interaction. Nature (London). 315: 207-210.

Favre-Bonvin J, N Arpin, and C Brevard. 1976. Structure de la mycosporine (P310). Can. J. Chem. 54: 1105-1113.

Favre-Bonvin J, J Bernillon, N Salin, and N Arpin. 1987. Biosynthesis of mycosporines: mycosporine-glutaminol in *Trichotecium roseum*. Phytochem. 26: 2509-2514.

Fitt WK. 1985. Chemosensory responses of the symbiotic dinoflagellate *Symbiodinium microadriaticum* (Dinophyceae). J. Phycol. 21: 62-67.

Fitt WK and ME Warner. 1995. Bleaching patterns of four species of Caribbean reef corals. Biol. Bull. 189: 298-307.

Fitt WK, HJ Spero, J Halas, MW White, and JW Porter. 1993. Recovery of the coral *Montastraea annularis* in the Florida Keys after the 1987 Caribbean "bleaching event". Coral Reefs. 12: 57-64.

Fitt WK, FK McFarland, ME Warner, and GC Chilcoat. 2000. Seasonal patterns of tissue biomass and densities of symbiotic dinoflagellates in reef corals and relation to coral bleaching. Limnol. Oceanogr. 45(3): 677-685.

Fleischmann EM. 1989. The measurement and penetration of ultraviolet radiation into tropical marine water. Limnol. Oceanogr. 34(8): 1623-1629.

Frederick JE, HE Snell, and EK Haywood. 1989. Solar ultraviolet radiation at the Earth's surface. Photochem. Photobiol. 50: 443-450.

Fridovich I. 1986. Biological effects of the superoxide radical. Arch. Biochem. Biophys. 247: 1-11.

Frank HA, A Cua, V Chynwat, A Young, D Gosztola, and MR Wasielewski. 1996. The lifetimes and energies of the first excited singlet states of diadinoxanthin and diatoxanthin: the role of these molecules in excess energy dissipation in algae. Biochim. Biophys. Acta. 1277: 243-252.

García-Pichel F. 1994. A model for internal self-shading in planktonic organisms and its implications for the usefulness of ultraviolet sunscreens. Limnol. Oceanogr. 39(7): 1704-1717.

García-Pichel F and RW Castenholz. 1993. Occurrence of UV-absorbing, mycosporine-like compounds among cyanobacterial isolates and an estimate of their screening capacity. Appl. Environ. Microbiol. 59(1): 163-169.

García-Pichel F, CE Wingard, and RW Castenholz. 1993. Evidence regarding the UV sunscreen role of a mycosporine-like compound in the cyanobacterium *Gloeocapsa* sp. Appl. Environ. Microbiol. 59(1): 170-176.

Gattuso J-P, D Allemand and M Frankignoulle. 1999. Photosynthesis and calcification at cellular, organismal and community levels in coral reefs: a review on interactions and control by carbonate chemistry. Am. Zool. 39: 160-183.

Gil-Turnes S and J Corredor. 1981. Studies of photosynthetic pigments of zooxanthellae in Caribbean hermatypic corals. Proc. 4$^{th}$ Int. Coral Reef. Symp. Manila. 2: 51-54.

Gladfelter EH. 1982. Skeletal development in *Acropora cervicornis*: I. Patterns of calcium carbonate accretion in the axial corallite. Coral Reefs. 1: 45-51.

Gladfelter EH. 1983. Skeletal development in *Acropora cervicornis*: II. Diel patterns of calcium carbonate accretion. Coral Reefs. 2: 91-100.

Gladfelter EH. 1984. Skeletal development in *Acropora cervicornis*: III. A comparison of monthly rates of linear extension and calcium carbonate accretion measured over a year. Coral Reefs. 3: 51-57.

Gleason DF. 1993. Differential effects of ultraviolet radiation on green and brown morphs of the Caribbean coral *Porites astreoides*. Limnol. Oceanogr. 38(7): 1452-1463.

Gleason DF and GM Wellington. 1993. Ultraviolet radiation and coral bleaching. Nature. 365: 836-838.

Gleason DF and GM Wellington. 1995. Variation in UVB sensitivity of planula larvae of the coral *Agaricia agaricites* along a depth gradient. Mar. Biol. 123: 693-703.

Glynn PW. 1993. Coral reef bleaching: ecological perspectives. Coral Reefs. 12: 1-17.

Glynn PW. 1996. Coral reef bleaching: facts, hypothesis and implications. Global Change Biol. 2: 495-509.

Goenaga C, VP Vicente, and RA Armstrong. 1989. Bleaching induced mortalities in reef corals from La Parguera, Puerto Rico: A precursor of change in the community structure of coral reefs? Carib. J. Sci. 25(1-2): 59-65.

Goreau TF. 1961. On the relation of calcification to primary productivity in reef building organisms. In: Lenhoff HH and WF Loomis (eds.) The biology of *Hydra* and some other coelenterates. University of Miami, Florida. pp. 269-285.

Goreau TJ and NI Goreau. 1973. The ecology of Jamaican coral reefs. II. Geomorphology, zonation and sedimentary phases. Bull. Mar. Sci. 23(2): 399-469.

Goreau TJ and AH Macfarlane. 1990. Reduced growth rate of *Montastraea annularis* following the 1987-1988 coral-bleaching event. Coral Reefs. 8: 211-215.

Grant, PT, C Middleton, PA Plack, and RH Tomson. 1985. The isolation of four aminocyclohexemines (mycosporines) and a structurally related derivative of cyclohexane-1,3-dione (gadusol) from the brine shrimp *Artemia*. Comp. Biochem. Physiol. 80B: 755-759.

Gröniger A, C Hallier, and DP Häder. 1999. Influence of UV radiation and visible light on *Porphyra umbilicalis*: photoinhibition and MAA concentration. J. Appl. Phycol. 11: 437-445.

Grottoli-Everett AG and IB Kuffner. 1995. Uneven bleaching within colonies of the Hawaiian coral *Montipora verrucosa*. In: Gulko D and PL Jokiel (eds) Ultraviolet Radiation and Coral Reefs. HIMB Tech. Report #41. UNIHI-Sea Grant-CR-95-03. pp.115-120.

Grunwald DJ and G Streisinger. 1992. Induction of mutations in the zebrafish with ultraviolet light. Genet. Res. 59(2): 93-101.

Gulko D. 1995. Effect of ultraviolet radiation on fertilization and production of planula larvae in the Hawaiian coral *Fungia scutaria*. In: Gulko D and PL Jokiel (eds) Ultraviolet Radiation and Coral Reefs. HIMB Tech. Report #41. UNIHI-Sea Grant-CR-95-03. pp. 135-147.

Häder DP, HD Kumar, RC Smith, and RC Worrest. 2003. Aquatic ecosystems: effects of solar ultraviolet radiation and interactions with other climatic change factors. Photochem. Photobiol. Sci. 2: 39-50.

Hager A. 1980. The reversible light-induced conversions of xanthophylls in the chloroplast. In: Czygan FC (ed.) Pigments in plants. Gustav-Fischer-Verlag. Stuttgart. p. 57-79.

Halliwell AG and JMC Gutteridge. 1999. Free radicals in biology and medicine. Oxford Science Publications. London. 936 pp.

Harrison PL and CC Wallace. 1990. Reproduction, dispersal and recruitment of scleractinian corals. In: Dubinsky Z (ed.) Ecosystems of the World: Coral Reefs. Elsevier. Amsterdam, The Netherlands. pp. 133-207.

Hoegh-Guldberg O. 1999. Climate change, coral bleaching and the future of the world's coral reefs. Mar. Freshwater Res. 50: 839-866.

Hoegh-Guldberg O and GJ Smith. 1989. The effect of sudden changes in temperature, light and salinity on the population density and export of zooxanthellae from the reef corals *Stylophora pistillata* Esper and *Seriatopora hystrix* Dana. J. Exp. Mar. Biol. Ecol. 129: 279-303.

Ishikura M, C Kato, and T Maruyama. 1997. UV-absorbing substances in zooxanthellate and azooxanthellate clams. Mar. Biol. 128: 649-655.

Jeffrey SW, R Douce, and AA Benson. 1974. Carotenoid transformations in the chloroplast envelope. Proc. Nat. Acad. Sci. USA. 71(3): 807-810.

Jeffrey SW, RFC Mantoura, and SW Wright. 1997. Phytoplankton pigments in oceanography: guidelines to modern methods. UNESCO Publishing. Paris. 661 pp.

Jeffrey SW, Sielicki M and FT Haxo. 1975. Chloroplast pigment patterns in dinoflagellates. J. Phycol. 11: 374-384.

Jeffrey SW, SW Wright, and M Zapata. 1999. Recent advances in HPLC pigments analysis of phytoplankton. Mar. Freshwater Res. 50: 879-896.

Jerlov NG. 1950. Ultraviolet radiation in the sea. Nature. 166: 111.

Jerlov NG. 1951. Optical studies of ocean water. Rep. Swedish Deep-Sea Exped. 3: 1-59.

Jerlov NG. 1968. Optical Oceanography. Elsevier. Amsterdam. 194 pp.

Johansen JE, WA Svec, and S Liaaen-Jensen. 1974. Carotenoids of the Dinophyceae. Phytochemistry. 13: 2261-2271.

Jokiel, PL. 1980. Solar ultraviolet radiation and coral reef epifauna. Science. 207: 1069-1071.

Jokiel PL. 1985. Lunar periodicity of planulae release in the reef coral *Pocillopora damicornis* in relation to various environmental factors. 5th Int. Coral Reef Congress. Tahiti. 4: 307-312.

Jokiel PL and RH York Jr. 1982. Solar ultraviolet photobiology of the reef coral *Pocillopora damicornis* and symbiotic zooxanthellae. Bull. Mar. Sci. 32(1): 301-315.

Jokiel PL and RH York Jr. 1984. Importance of ultraviolet radiation in photoinhibition of microalgal growth. Limnol. Oceanogr. 29(1): 192-199.

Jones RJ. 1997a. Zooxanthellae loss as a bioassay for assessing stress in corals. Mar. Ecol. Prog. Ser. 149: 163-171.

Jones RJ. 1997b. Changes in zooxanthellar densities and chlorophyll concentrations: in corals during and after a bleaching event. Mar. Ecol. Prog. Ser. 158: 51-59.

Karentz D, FS McEuen, MC Land, and WC Dunlap. 1991. Survey of mycosporine-like amino acid compounds in Antartic marine organisms: potential protection from ultraviolet exposure. Mar. Biol. 108: 157-166.

Karsten U, T Sawall, J West, and C Wiencke. 2000. Ultraviolet sunscreen compounds in epiphytic red algae from mangroves. Hydrobiol. 432: 159-171.

Karsten U, T Sawall, D Hanelt, K Bischof, FL Figueroa, A Flores-Moya and C Wiencke. 1998. An inventory of UV-absorbing mycosporine-like amino acids in macroalgae from polar to warm-temperate regions. Botanica Marina. 41: 443-453.

Kojis BL and NJ Quinn. 1984. Seasonal and depth variation in fecundity of *Acropora palifera* at two reefs in Papua New Guinea. Coral Reefs. 3: 165-172.

Kinzie III RA. 1993. Effects of ambient levels of solar ultraviolet radiation on zooxanthellae and photosynthesis of the reef coral *Montipora verrucosa*. Mar. Biol. 116: 319-327.

Kinzie III RA, M Takayama, SR Santos, and MA Coffroth. 2001. The adaptive bleaching hypothesis: experimental tests of critical assumptions. Biol. Bull. 200: 51-58.

Kirk JTO. 1994. Light and photosynthesis in aquatic ecosystems. Cambridge Univ. Press. New York. 509pp.

Kleypas JA, RW Buddemeier, and J-P Gattuso. 2001. The future of coral reefs in an age of global change. Int. J. Earth Sciences. 90: 426-437.

Kleypas JA, RW Buddemeier, D Archer, J-P Gattuso, C Langdon, and BN Opdyke. 1999. Geochemical consequences of increased atmospheric carbon dioxide on coral reefs. Science. 284: 118-120.

Krinsky NI. 1971. Function (carotenoids). In: Isler O (ed) Carotenoids. Halsted Press, John-Wiley. New York. pp. 669-716.

Krupp DA. 1983. Sexual reproduction and early development of the solitary coral *Fungia scutaria* (Anthozoa: Scleractinia). Coral Reefs. 2: 159-164.

Krupp DA and J Blanck. 1995. Preliminary report on the occurrence of mycosporine-like amino acids in the eggs of the Hawaiian scleractinian corals *Montipora verrucosa* and *Fungia scutaria*. In: Gulko D and PL Jokiel (eds) Ultraviolet Radiation and Coral Reefs. HIMB Tech. Report #41. UNIHI-Sea Grant-CR-95-03. pp. 129-134.

Kuffner IB. 2001a. Effects of ultraviolet radiation and water motion on the reef coral *Porites compressa* Dana: a flume experiment. Mar. Biol. 138: 467-476.

Kuffner IB. 2001b. Effects of ultraviolet (UV) radiation on larval settlement of the reef coral *Pocillopora damicornis*. Mar. Ecol. Prog. Ser. 217: 251-261.

Kuffner IB, ME Ondrusek, and MP Lesser. 1995. Distribution of mycosporine-like amino acids in the tissues of Hawaiian Scleractinia: a depth profile. In: Gulko D and PL Jokiel (eds) Ultraviolet Radiation and Coral Reefs. HIMB Tech. Report #41. UNIHI-Sea Grant-CR-95-03. pp. 77-85.

LaJeunesse TC. 2002. Diversity and community structure of symbiotic dinoflagellates from Caribbean coral reefs. Mar. Biol. 141: 387-400.

LaJeunesse TC, WKW Loh, R van Woesik, O Hoegh-Guldberg, GW Schmidt, and WK Fitt. 2003. Low symbiont diversity in southern Great Barrier Reef corals, relative to those of the Caribbean. Limnol. Oceanogr. 48(5): 2046-2054.

Lamberts AE. 1978. Coral growth: alizarin method. In: Stoddart DR and RE Johannes (eds.) Coral reef: research methods. UNESCO. p. 523-527.

Lasker HR. 2003. Zooxanthella densities within a Caribbean octocoral during bleaching and non-bleaching years. Coral Reefs. 22: 23-26.

Leach CM. 1965. Ultraviolet absorbing substances associated with light-induced sporulation in fungi. Can. J. Bot. 43: 185-200.

Leclercq N, J-P Gattuso, and J Jaubert. 2002. Primary production, respiration, and calcification of a coral reef mesocosm under increased $CO_2$ partial pressure. Limnol. Oceanogr. 47(2): 558-564.

Leder JJ, AM Szmant, and PK Swart. The effect of prolonged “bleaching” on skeletal banding and stable isotopic composition in *Montastraea annularis*. Coral Reefs. 10: 19-27.

Lesser 1995. General overview of instrumentation, experimental methods, and attenuation of UV radiation in natural waters. In: Gulko D and PL Jokiel (eds) Ultraviolet Radiation and Coral Reefs. HIMB Tech. Report #41. UNIHI-Sea Grant-CR-95-03. pp. 15-18.

Lesser MP. 1996. Exposure of symbiotic dinoflagellates to elevated temperatures and ultraviolet radiation causes oxidative stress and inhibits photosynthesis. Limnol. Oceanogr. 41: 271-283.

Lesser MP. 1997. Oxidative stress causes coral bleaching during exposure to elevated temperatures. Coral Reefs. 16: 187-192.

Lesser MP and TM Barry. 2003. Survivorship, development, and DNA damage in echinoderm embryos and larvae exposed to ultraviolet radiation (290-400 nm). J. Exp. Mar. Biol. Ecol. 292: 75-91.

Lesser MP and S Lewis. 1996. Action spectrum for the effects of UV radiation on photosynthesis in the hermatypic coral *Pocillopora damicornis*. Mar. Ecol. Prog. Ser. 134: 171-177.

Lesser MP and JM Shick. 1989. Effects of irradiance and ultraviolet radiation on photoadaptation in the zooxanthellae of *Aiptasia pallida*: primary production, photoinhibition, and enzymatic defenses against oxygen toxicity. Mar. Biol. 102: 243-255.

Lesser MP, WR Stochaj, DW Tapley, and JM Shick. 1990. Bleaching in coral reef anthozoans: effects of irradiance, ultraviolet radiation and temperature on the activities of protective enzymes against active oxygen. Coral Reefs. 8: 225-232.

Lesser MP, C Mazel, D Phinney, and CS Yentsch. 2000. Light absorption and utilization by colonies of the congeneric hermatypic corals *Montastraea faveolata* and *Montastraea cavernosa*. Limnol. Oceanogr. 45(1): 76-86.

Lewis S. 1995. Response of a Pacific stony coral to short-term exposure of ultraviolet and visible light. In: Gulko D and PL Jokiel (eds) Ultraviolet Radiation and Coral Reefs. HIMB Tech. Report #41. UNIHI-Sea Grant-CR-95-03. pp. 89-106.

Liaaen-Jensen S. 1978. Marine carotenoids. In: Scheuer PJ (ed.) Marine natural products. Academic Press. New York. p. 1-73.

Madronich S, RL McKenzie, LO Björn, and MM Caldwell. 1995. Changes in ultraviolet radiation reaching the Earth's surface. Ambio. 24: 143-152.

Madronich S, RL McKenzie, LO Björn, and MM Caldwell. 1998. Changes in biologically active ultraviolet radiation reaching the Earth's surface. Photochem. Photobiol. B. 46: 5-19.

Marsh JA (1970) Primary productivity of reef building calcareous red algae. Ecology 51: 255-263.

Marubini F, C Ferrier-Pages, and JP Cuif. 2003. Suppression of skeletal growth in scleractinian corals by decreasing ambient carbonate-ion concentration: A cross-family comparison. Proc. R. Soc. London Ser B. 270: 179-184.

Masuda K, M Goto, T Maruyama, and S Miyachi. 1993. Adaptation of solitary corals and their zooxanthellae to low light and UV radiation. Mar. Biol. 117: 685-691.

Mazel CH, MP Lesser, MY Gorbunov, TM Barry, JH Farrell, KD Wyman and PG Falkowski. 2003. Green-fluorescent proteins in Caribbean corals. Limnol. Oceanogr. 48(1, part 2): 402-411.

McConnaughey TA. 1989. Biomineralization mechanisms. In: Crick RE (ed.) Origin, evolution, and modern aspects of biomineralization in plants and animals. Plenum Press. New York. pp. 57-73.

McConnaughey TA. 1994. Calcification, photosynthesis, and global carbon cycles. In: Doumenge F (ed.) Past and present biomineralization processes. Considerations about the carbonate cycle. IUCN-COE Workshop. Monaco. pp. 137-161.

McConnaughey TA, WH Adey, and AM Small. 2000. Community and environmental influences on reef coral calcification. Limnol. Oceanogr. 45(7): 1667-1671.

McNeil BI, RJ Matear, and DJ Barnes. 2004. Coral reef calcification and climate change: the effect of ocean warming. Geophys. Res. Lett. 31. L22309. doi:10.1029/2004GL021541.

Meyer JL and ET Schultz. 1985. Tissue condition and growth rate of corals associated with schooling fish. Limnol. Oceanogr. 30: 157-166.

Michalek-Wagner K. 2001. Seasonal and sex-specific variations in levels of photo-protecting mycosporine-like amino acids (MAAs) in soft corals. Mar. Biol. 139: 651-660.

Michalek-Wagner K and BL Willis. 2001a. Impacts of bleaching on the soft coral *Lobophytum compactum*. I. Fecundity, fertilization and offspring viability. Coral Reefs. 19: 231-239.

Michalek-Wagner K and BL Willis. 2001b. Impacts of bleaching on the soft coral *Lobophytum compactum*. II. Biochemical changes in adults and their eggs. Coral Reefs. 19: 240-246.

Moisan TA and BG Mitchell. 2001. UV absorption by mycosporine-like amino acids in *Phaeocystis antarctica* Karsten induced by photosynthetically available radiation. Mar. Biol. 138: 217-227.

Molina MJ and FS Rowland. 1974. Stratospheric sink for chlorofluoromethanes: chlorine atom-catalyzed destruction of ozone. Nature. 249: 810-812.

Müller A, MK Gagan, and JM Lough. 2004. Effect of early marine diagenesis on coral reconstructions of surface-ocean $^{13}C/^{12}C$ and carbonate saturation state. Global Biogeochem. Cycles. 18: GB1033. doi:10.1029/2003GB002112.

Muscatine L. 1990. The role of symbiotic algae in carbon and energy flux in reef corals. In: Dubinsky Z (ed.) Ecosystems of the World: Coral Reefs. Elsevier. Amsterdam, The Netherlands. pp. 75-86.

Muscatine L and E Cernichiari. 1969. Assimilation of photosynthetic products of zooxanthellae by a reef coral. Biol. Bull. Mar. Biol. Lab. Woods Hole. 137: 506-523.

Muscatine L, LR McCloskey, and RE Marian. 1981. Estimating the daily contribution of carbon from zooxanthellae to coral animal respiration. Limnol. Oceanogr. 26(4): 601-611.

Muszynski FZ. 1997. UV radiation-induced alterations in the pigment characteristics of the hermatypic coral *Montastraea faveolata* (Ellis and Solander, 1786) in southwest, Puerto Rico. MS Thesis. Univ. of PR. 41pp.

Nakamura H, J Kobayashi, and Y Hirata. 1982. Separation of mycosporine-like amino acids in marine organisms using reversed phased high performance liquid chromatography. J. Chromatogr. 250: 113-118.

Oliver JK. 1984. Intra-colony variation in the growth of *Acropora formosa*: Extension rates and skeletal structure of white (zooxanthellae-free) and brown-tipped branches. Coral Reefs. 3: 139-147.

Omori M, H Fukami, H Kobinata and M Hatta. 2001. Significant drop of fertilization of *Acropora* corals in 1999: an after-effect of heavy coral bleaching? Limnol. Oceanogr. 46(3): 704-706.

Orce VL and EW Helbling. 1997. Latitudinal UVR-PAR measurements in Argentina: extent of the ozone hole. Global Planet. Change. 15: 113-121.

Orr JC, VJ Fabry, O Aumont, L Bopp, SC Doney, RA Feely, A Gnanadesikan, N Gruber, A Ishida, F Joos, RM Key, K Lindsay, E Maier-Reimer, R Matear, P Monfray, A Mouchet, RG Najjar, GK Plattner, KB Rodgers, CL Sabine, JL Sarmiento, R Schlitzer, RD Slater, IJ Totterdell, MF Weirig, Y Yamanaka, and A Yool. 2005. Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. Nature. 437: 681-686.

Ott RL and M Longnecker. 2001. An introduction to statistical methods and data analysis. Duxbury, California, USA. 1152 pp.

Peak MJ and JG Peak. 1990. Hydroxyl radical quenching agents protect against DNA breakage caused by both 365-nm and gamma radiation. Photochem. Photobiol. 51: 649-652.

Pelejero C, E Calvo, MT McCulloch, JF Marshall, MK Gagan, JM Lough, and BN Opdyke. 2005. Preindustrial to modern interdecadal variability in coral reef pH. Science. 309: 2204-2207.

Peters EC. 1984. A survey of cellular reactions to environmental stress and disease in Caribbean scleractinian corals. Hegol. Wiss. Meeresunters. 37: 113-137.

Reynaud S, N Leclercq, S Romaine-Lioud, C Ferrier-Pages, J Jaubert, and JP Gattuso. 2003. Interacting effects of CO2 partial pressure and temperature on photosynthesis and calcification in a scleractinian coral. Global Change Biol. 9: 1660-1668.

Richardson LL. 1998. Coral diseases: what is really known? Trends Ecol. Conserv. 13: 438-443.

Richmond RH and CL Hunter. 1990. Reproduction and recruitment of corals: comparisons among the Caribbean, the tropical Pacific, and the Red Sea. Mar. Ecol. Prog. Ser. 60: 185-203.

Rinchevich B and Y Loya. 1979. The reproduction of the Red Sea coral *Stylophora pistillata*. II. Synchronization in breeding and seasonality of planulae shedding. Mar. Ecol. Prog. Ser. 1: 145-152.

Roberfroid M and PB Calderon. 1995. Free radicals and oxidation phenomena in biological systems. Université Catholique de Louvain, Brussels, Belgium. Marcel Dekker, Inc. NY. 270pp.

Rogers CS. 1990. Responses of coral reefs and reef organisms to sedimentation. Mar. Ecol. Prog. Ser. 62: 185-202.

Rosenberg E and Y Ben-Haim. 2002. Microbial diseases of corals and global warming. Eviron. Mircobiol. 4(6): 318-326.

Rowan R. 1998. Diversity and ecology of zooxanthellae on coral reefs. J. Phycol. 34: 407-417.

Rowan R. 2004. Thermal adaptation in reef coral symbionts. Nature. 430:742.

Rowan R and DA Powers. 1991. Molecular genetic identification of symbiotic dinoflagellates (zooxanthellae). Mar. Ecol. Prog. Ser. 71: 65-73.

Rowan R and N Knowlton. 1995. Intraspecific diversity and ecological zonation in coral-algal symbiosis. Proc. Natl. Acad. Sci. USA. 92: 2850-2853.

Rozema J, LO Björn, JF Bornman, A Gaberščik, D-P Häder, T Trošt, M Germ, M Klisch, A Gröniger, RP Sinha, M Lebert, Y-Y He, R Buffoni-Hall, NVJ de Bakker, J van de Staaij, and BB Meijkamp. 2002. The role of UV-B radiation in aquatic and terrestrial ecosystems – an experimental and functional analysis of the evolution of UV-absorbing compounds. J. Photochem. Photobiol. B. 66: 2-12.

Salih A, O Hoegh-Guldberg, and G Cox. 1998b. Photoprotection of symbiotic dinoflagellates by fluorescent pigments in reef corals. In: Greenwood JG and NJ Hall (eds) Proc. Australian Coral Reef Soc. 75th Anniversary Conference. Heron Island. pp. 217-230.

Salih A, A Larkum, G Cox, M Kuhl and O Hoegh-Guldberg. 2000. Fluorescent pigments in corals are photoprotective. Nature. 408: 850-853.

Savage AM, MS Goodson, S Visram, H Trapido-Rosenthal, J Wiedenmann, and AE Douglas. 2002a. Molecular diversity of symbiotic algae at the latitudinal margins of their distribution: dinoflagellates of the genus *Symbiodinium* in corals and sea anemones. Mar. Ecol. Prog. Ser. 244: 17-26.

Scelfo GM. 1988. Ultraviolet-B absorbing compounds in *Anthopleura elegantissima*. Am. Zool. 28: 105A.

Schlichter D and HW Fricke. 1990. Coral host improves photosynthesis of endosymbiotic algae. Naturwissenschaften. 7: 447-450.

Shashar N, AT Banaszak, MP Lesser, and D Amrami. 1997. Coral endolithic algae: life in a protected environment. Pacific Science. 51(2): 167-173.

Shibata K. 1969. Pigments and a UV-absorbing substance in corals and a blue-green alga living in the Great Barrier Reef. Plant Cell Physiol. 10: 325-335.

Shick JM, WC Dunlap, BE Chalker, AT Banaszak, and TK Rosenzweig. 1992. Survey of ultraviolet radiation-absorbing mycosporine-like amino acids in organs of coral reef holothuroids. Mar. Ecol. Prog. Ser. 90: 139-148.

Shick JM, MP Lesser, WC Dunlap, WR Stochaj, BE Chalker, and J Wu Won. 1995. Depth-dependent responses to solar ultraviolet radiation and oxidative stress in the zooxanthellate coral *Acropora microphthalma*. Mar. Biol. 122: 41-51.

Shick JM, S Romaine-Lioud, C Ferrier-Pagès, and J-P Gattuso. 1999. Ultraviolet-B radiation stimulates shikimate pathway-dependent accumulation of mycosporine-like amino acids in the coral *Stylophora pistillata* despite decreases in its population of symbiotic dinoflagellates. Limnol. Oceanogr. 44(7): 1667-1682.

Shinn EA. 1966. Coral growth-rate, an environmental indicator. J. Paleo. 40(2): 233-241.

Siebeck O. 1981. Photoreactivation and depth-dependent U.V. tolerance in reef coral in the Great Barrier Reef/Australia. Naturwissenschaften. 67: 426-427.

Siebeck O. 1988. Experimental investigation of UV tolerance in hermatypic corals (Scleractinia). Mar. Ecol. Prog. Ser. 43: 95-103.

Smith RC. 1989. Ozone, middle ultraviolet radiation, and the aquatic environment. Photochem. Photobiol. 50: 459-468.

Smith RC and KS Baker. 1979. Penetration of UV-B and biologically effective dose-rates in natural waters. Photochem. Photobiol. 29: 311-323.

Smith SV and RW Buddemeier. 1992. Global change and coral reef ecosystems. Annu. Rev. Ecol. Syst. 23: 89-118.

Steiner SCC. 1995. Spawning in scleractinian corals from SW Puerto Rico (West Indies). Bull Mar. Sci. 56(3): 899-902.

Stimson J, K Sakai, and H Sembali. 2002. Interspecific comparison of the symbiotic relationship in corals with high and low rates of bleaching-induced mortality. Coral Reefs. 21: 409-421.

Stochaj WR, WC Dunlap, and JM Shick. 1994. Two new UV-absorbing mycosporine-like amino acids from the sea anemone *Anthopleura elegantissima* and the effects of zooxanthellae and spectral irradiance on chemical composition and content. Mar. Biol. 118: 149-156.

Soong K. 1991. Sexual reproductive patterns of shallow-water reef corals in Panama. Bull. Mar. Sci. 49(3): 832-846.

Soong, K. 1993. Colony size as a species character in massive reef corals. Coral Reefs. 12: 77-83.

Szmant AM. 1986. Reproductive ecology of Caribbean reef corals. Coral Reefs. 5: 43-54.

Szmant AM. 1991. Sexual reproduction by the Caribbean reef corals *Montastraea annularis* and *M. cavernosa*. Mar. Ecol. Prog. Ser. 74: 13-25.

Szmant AM and NJ Gassman. 1990. The effects of prolonged “bleaching” on the tissue biomass and reproduction of the reef coral *Montastraea annularis*. Coral Reefs. 8: 217-224.

Szmant-Froelich AM, M Reutter, and L Riggs. 1985. Sexual reproduction of *Favia fragum* (Esper): Lunar patterns of gametogenesis, embryogenesis and planulation in Puerto Rico. Bull. Mar. Sci. 37(3): 880-892.

Takano S, D Uemura, and Y Hirata. 1978a. Isolation and structure of a new amino acid, palythine, from the zoanthid *Palythoa tuberculosa*. Tetrahedron Letters. 26: 2299-2300.

Takano S, D Uemura, and Y Hirata. 1978b. Isolation and structure of two new amino acids, palythinol and palythene, from the zoanthid *Palythoa tuberculosa*. Tetrahedron Letters. 49: 4909-4912.

Takano S, A Nakanishi, D Uemura, and Y Hirata. 1979. Isolation and structure of a 334nm UV-absorbing substance, porphyra-334 from the red alga *Porphyra tenera* Kjellman. Chem. Lett. 1979: 419-420.

Tartarotti B and R Sommaruga. 2002. The effect of different methanol concentrations and temperatures on the extraction of mycosporine-like amino acids (MAAs) in algae and zooplankton. Arch. Hydrobiol. 154(4): 691-703.

Teai T, JH Drollet, J-P Bianchini, A Cambon, and PMV Martin. 1997. Widespread occurrence of mycosporine-like amino acids in scleractinians from French Polynesia. Coral Reefs. 16: 169-176.

Teai T, JH Drollet, J-P Bianchini, A Cambon, and PMV Martin. 1998. Occurrence of ultraviolet radiation-absorbing mycosporine-like amino acids in coral mucus and whole corals of French Polynesia. Mar. Freshwater Res. 49: 127-132.

Toller WW, R Rowan and N Knowlton. 2001a. Zooxanthellae of the *Montastraea annularis* species complex: patterns of distribution of four taxa of *Symbiodinium* on different reefs and across depths. Biol. Bull. 201: 348-359.

Toller WW, R Rowan and N Knowlton. 2001b. Repopulation of zooxanthellae in the Caribbean corals *Montastraea annularis* and *M. faveolata* following experimental and disease-associated bleaching. Biol. Bull. 201: 360-373.

Tomasick and F Sanders. 1985. Effects of eutrophication on reef-building corals: growth rate of the reef-building coral *Montastraea annularis*. Mar. Biol. 87: 143-155.

Torres JL. 2001. Impacts of sedimentation on the growth rates of *Montastraea annularis* in southwest, Puerto Rico. Proceedings of the International Conference on Scientific Aspects of Coral Reef Assessment, Monitoring and Restoration (National Coral Reef Institute). Bull. Mar. Sci. 69(2): 631-637.

Torres JL and J Morelock. 2002. Effects of terrigenous sediments influx on the coral cover and linear extension rates of three Caribbean massive coral species. Carib. J. Sci. 38(3-4): 222-229.

Tyrrell RM. 1991. UVA (320-380nm) radiation as an oxidative stress. In: Sies H (ed) Oxidative stress: oxidants and antioxidants. Acad. Press. San Diego. pp. 57-83.

Vandermeulen JH, ND Davis, and L Muscatine. 1972. The effect of inhibitors of photosynthesis of zooxanthellae in corals and other invertebrates. Mar. Biol. 16: 185-191.

Vargas-Toledo W. 2002. Sexual reproduction in the Caribbean genus *Diploria*, in La Parguera, Puerto Rico. MS Thesis. Univ. of PR. 102 pp.

Villinski JT. 2003. Depth-dependent reproductive characteristics for the Caribbean reef-building coral *Montastraea faveolata*. Mar. Biol. 142: 1043-1053.

Ward S. 1995. Two patterns of energy allocation for growth, reproduction and lipid storage in the scleractinian coral *Pocillopora damicornis*. Coral Reefs. 14: 87-90.

Warner ME, WK Fitt, and GW Schmidt. 1999. Damage to photosystem II in symbiotic dinoflagellates: A determinant of coral bleaching. Proc. Natl. Acad. Sci. USA. 96: 8007-8012.

Warner ME, GC Chilcoat, FK McFarland, and WK Fitt. 2002. Seasonal fluctuations in the photosynthetic capacity of photosystem II in symbiotic dinoflagellates in the Caribbean reef-building coral *Montastraea*. Mar. Biol. 141: 31-38.

Wilkerson FP, D Kobayashi, and L. Muscatine. 1988. Mitotic index and size of symbiotic algae in Caribbean reef corals. Coral Reefs. 7: 29-36.

Wilkerson FP, Muller-Parker G, and L Muscatine. 1983. Temporal patterns of cell division in natural populations of endosymbiotic algae. Limnol. Oceanogr. 28: 1009-1014.

Wilkinson CR. 1999. Global and local threats to coral reef functioning and existence: review and predictions. Mar. Freshwater Res. 50: 867-878.

Wilkinson C, O Lindén, H Cesar, G Hodgson, J Rubens, and AE Strong. 1999. Ecological and socioeconomic impacts of 1998 coral mortality in the Indian Ocean: An ENSO impact and a warming of future change? Ambio. 28(2): 188-196.

Winter A, RS Appeldoorn, A Bruckner, EH Williams Jr., and C Goenaga. 1998. Sea surface temperatures and coral reef bleaching off La Parguera, Puerto Rico (northeastern Caribbean Sea). Coral Reefs. 17: 377-382.

Winters G, Y Loya, R Röttgers and S Beer. 2003. Photoinhibition in shallow-water colonies of the coral *Stylophora pistillata* as measured in situ. Limnol. Oceanogr. 48(4): 1388-1393.

Worrest RC, H Van Dyke, and BE Thomson. 1978. Impact of enhanced simulated solar ultraviolet radiation upon a marine community. Photochem. Photobiol. 27: 471-478.

Wright SW, SW Jeffrey, RFC Mantoura, CA Llewellyn, T Bjørnland, D Repeta, and N Welschmeyer. 1991. Improved HPLC method for the analysis of chlorophylls and carotenoids from marine phytoplankton. Mar. Ecol. Prog. Ser. 77: 183-196.

Wu Won JJ, JA Rideout, and BE Chalker. 1995. Isolation and structure of a novel mycosporine-like amino acid from the reef-building corals *Pocillopora damicornis* and *Stylophora pistillata*. Tetrahedron Letters. 36(29): 5255-5256.

Wyman KD, Z Dubinsky, JW Porter, and FG Falkowski. 1987. Light absorption and utilization among hermatypic corals: a study from Jamaica, West Indies. Mar. Biol. 96: 283-292.

Yonge CM. 1940. The biology of reef-building corals. Sci. Rep. Gt. Barrier Reef Exped: 1928-1929. Br. Mus. Nat. Hist. 1: 353-391.

# APPENDIX I

## SUMMARY OF STATISTICAL ANALYSES

### UV-E Experiment

Fully Nested Analysis of Variance for Linear extension rates of *A. cervicornis* (UV-E Experiment), using Adjusted SS for Tests

| Source | DF | Seq SS | Adj SS | Adj MS | F | P |
|---|---|---|---|---|---|---|
| Treatment | 2 | 0.256 | 0.256 | 0.128 | 26.36 | 0.000 |
| Replicates(Treatment) | 4 | 0.017 | 0.017 | 0.004 | 0.87 | 0.489 |
| Error | 35 | 0.170 | 0.170 | 0.005 | | |
| Total | 41 | 0.444 | | | | |

Fully Nested Analysis of Variance for skeletal densities of *A. cervicornis* (UV-E Experiment), using Adjusted SS for Tests

| Source | DF | Seq SS | Adj SS | Adj MS | F | P |
|---|---|---|---|---|---|---|
| Treatment | 2 | 0.022 | 0.022 | 0.011 | 0.32 | 0.732 |
| Replicates(Treatment) | 4 | 0.089 | 0.089 | 0.022 | 0.64 | 0.641 |
| Error | 35 | 1.226 | 1.226 | 0.035 | | |
| Total | 41 | 1.337 | | | | |

Fully Nested Analysis of Variance for Linear extension rates of *P. furcata* (UV-E Experiment), using Adjusted SS for Tests

| Source | DF | Seq SS | Adj SS | Adj MS | F | P |
|---|---|---|---|---|---|---|
| Treatment | 2 | 0.0106 | 0.0106 | 0.0053 | 13.47 | 0.000 |
| Replicates(Treatment) | 4 | 0.0006 | 0.0006 | 0.0002 | 0.39 | 0.811 |
| Error | 35 | 0.0138 | 0.0138 | 0.0004 | | |
| Total | 41 | 0.0250 | | | | |

Fully Nested Analysis of Variance for skeletal densities of *P. furcata* (UV-E Experiment), using Adjusted SS for Tests

| Source | DF | Seq SS | Adj SS | Adj MS | F | P |
|---|---|---|---|---|---|---|
| Treatment | 2 | 0.160 | 0.160 | 0.080 | 2.15 | 0.132 |
| Replicates(Treatment) | 4 | 0.111 | 0.111 | 0.028 | 0.75 | 0.566 |
| Error | 35 | 1.301 | 1.301 | 0.038 | | |
| Total | 41 | 1.572 | | | | |

Fully Nested Analysis of Variance for concentration of MAA's in *A. cervicornis* (UV-E Experiment), using Adjusted SS for Tests

| Source | DF | Seq SS | Adj SS | Adj MS | F | P |
|---|---|---|---|---|---|---|
| Treatment | 2 | 582186 | 582186 | 291093 | 342.06 | 0.000 |
| Replicates(Treatment) | 4 | 674 | 674 | 169 | 0.20 | 0.938 |
| Error | 35 | 29785 | 29785 | 851 | | |
| Total | 41 | 612645 | | | | |

Fully Nested Analysis of Variance for concentration of MAA's in *P. furcata* (UV-E Experiment), using Adjusted SS for Tests

| Source | DF | Seq SS | Adj SS | Adj MS | F | P |
|---|---|---|---|---|---|---|
| Treatment | 2 | 77985 | 77985 | 38993 | 272.73 | 0.000 |
| Replicates(Treatment) | 4 | 86 | 86 | 21 | 0.15 | 0.962 |
| Error | 35 | 5004 | 5004 | 143 | | |
| Total | 41 | 83075 | | | | |

Fully Nested Analysis of Variance for concentration of photosynthetic pigments per coral tissue area in *A. cervicornis* (UV-E Experiment), using Adjusted SS for Tests

| Source | DF | Seq SS | Adj SS | Adj MS | F | P |
|---|---|---|---|---|---|---|
| Treatment | 2 | 653.19 | 653.19 | 326.59 | 34.60 | 0.000 |
| Replicates(Treatment) | 4 | 24.36 | 24.36 | 6.09 | 0.65 | 0.634 |
| Error | 35 | 330.41 | 330.41 | 9.44 | | |
| Total | 41 | 1007.96 | | | | |

Fully Nested Analysis of Variance for concentration of photosynthetic pigments per zooxanthellae cell in *A. cervicornis* (UV-E Experiment), using Adjusted SS for Tests

| Source | DF | Seq SS | Adj SS | Adj MS | F | P |
|---|---|---|---|---|---|---|
| Treatment | 2 | 20.80 | 20.80 | 10.40 | 0.89 | 0.419 |
| Replicates(Treatment) | 4 | 6.55 | 6.55 | 1.64 | 0.14 | 0.966 |
| Error | 35 | 408.65 | 408.65 | 11.68 | | |
| Total | 41 | 436.01 | | | | |

Fully Nested Analysis of Variance for concentration of photosynthetic pigments per coral tissue area in *P. furcata* (UV-E Experiment), using Adjusted SS for Tests

| Source | DF | Seq SS | Adj SS | Adj MS | F | P |
|---|---|---|---|---|---|---|
| Treatment | 2 | 11683.8 | 11683.8 | 5841.9 | 33.17 | 0.000 |
| Replicates(Treatment) | 4 | 199.6 | 199.6 | 49.9 | 0.28 | 0.887 |
| Error | 35 | 6164.0 | 6164.0 | 176.1 | | |
| Total | 41 | 18047.4 | | | | |

Fully Nested Analysis of Variance for concentration of photosynthetic pigments per zooxanthellae cell in *P. furcata* (UV-E Experiment), using Adjusted SS for Tests

| Source | DF | Seq SS | Adj SS | Adj MS | F | P |
|---|---|---|---|---|---|---|
| Treatment | 2 | 38770.7 | 38770.7 | 19385.4 | 33.87 | 0.000 |
| Replicates(Treatment) | 4 | 5019.0 | 5019.0 | 1254.8 | 2.19 | 0.090 |
| Error | 35 | 20030.6 | 20030.6 | 572.3 | | |
| Total | 41 | 63820.3 | | | | |

Fully Nested Analysis of Variance for zooxanthellae density in *A. cervicornis* (UV-E Experiment), using Adjusted SS for Tests

| Source | DF | Seq SS | Adj SS | Adj MS | F | P |
|---|---|---|---|---|---|---|
| Treatment | 2 | 1.004 | 1.004 | 0.502 | 1.87 | 0.169 |
| Replicates(Treatment) | 4 | 0.529 | 0.529 | 0.132 | 0.49 | 0.741 |
| Error | 35 | 9.403 | 9.403 | 0.269 | | |
| Total | 41 | 10.937 | | | | |

Fully Nested Analysis of Variance for the Mitotic Index of zooxanthellae in *A. cervicornis* (UV-E Experiment), using Adjusted SS for Tests

| Source | DF | Seq SS | Adj SS | Adj MS | F | P |
|---|---|---|---|---|---|---|
| Treatment | 2 | 0.0011 | 0.0011 | 0.00056 | 3.98 | 0.028 |
| Replicates(Treatment) | 4 | 0.0004 | 0.0004 | 0.00011 | 0.79 | 0.539 |
| Error | 35 | 0.0049 | 0.0049 | 0.00014 | | |
| Total | 41 | 0.0065 | | | | |

Fully Nested Analysis of Variance for zooxanthellae density in *P. furcata* (UV-E Experiment), using Adjusted SS for Tests

| Source | DF | Seq SS | Adj SS | Adj MS | F | P |
|---|---|---|---|---|---|---|
| Treatment | 2 | 0.131 | 0.131 | 0.0657 | 2.07 | 0.142 |
| Replicates(Treatment) | 4 | 0.098 | 0.098 | 0.0245 | 0.77 | 0.552 |
| Error | 35 | 1.112 | 1.112 | 0.0318 | | |
| Total | 41 | 1.341 | | | | |

Fully Nested Analysis of Variance for the Mitotic Index of zooxanthellae in *P. furcata* (UV-E Experiment), using Adjusted SS for Tests

| Source | DF | Seq SS | Adj SS | Adj MS | F | P |
|---|---|---|---|---|---|---|
| Treatment | 2 | 0.0001 | 0.0001 | 0.00007 | 1.41 | 0.259 |
| Replicates(Treatment) | 4 | 0.0003 | 0.0003 | 0.00007 | 1.43 | 0.245 |
| Error | 35 | 0.0017 | 0.0017 | 0.00005 | | |
| Total | 41 | 0.0021 | | | | |

**UV-S Experiment**

Fully Nested Analysis of Variance for Linear extension rates of *A. cervicornis* (UV-S Experiment), using Adjusted SS for Tests

| Source | DF | Seq SS | Adj SS | Adj MS | F | P |
|---|---|---|---|---|---|---|
| Treatment | 5 | 2.583 | 2.534 | 0.507 | 36.15 | 0.000 |
| Replicates(Treatment) | 12 | 0.005 | 0.005 | 0.0004 | 0.03 | 1.000 |
| Error | 42 | 0.589 | 0.589 | 0.014 | | |
| Total | 59 | 3.177 | | | | |

Fully Nested Analysis of Variance for skeletal densities of *A. cervicornis* (UV-S Experiment), using Adjusted SS for Tests

| Source | DF | Seq SS | Adj SS | Adj MS | F | P |
|---|---|---|---|---|---|---|
| Treatment | 5 | 1.529 | 1.529 | 0.306 | 5.11 | 0.001 |
| Replicates(Treatment) | 12 | 0.197 | 0.197 | 0.017 | 0.27 | 0.990 |
| Error | 42 | 2.512 | 2.512 | 0.060 | | |
| Total | 59 | 4.238 | | | | |

Fully Nested Analysis of Variance for Linear extension rates of *P. furcata* (UV-S Experiment), using Adjusted SS for Tests

| Source | DF | Seq SS | Adj SS | Adj MS | F | P |
|---|---|---|---|---|---|---|
| Treatment | 2 | 0.000049 | 0.000049 | 0.000024 | 47.21 | 0.000 |
| Replicates(Treatment) | 6 | 0.000003 | 0.000003 | 0.000000 | 1.07 | 0.415 |
| Error | 18 | 0.000009 | 0.000009 | 0.000000 | | |
| Total | 26 | 0.000062 | | | | |

Fully Nested Analysis of Variance for skeletal densities of *P. furcata* (UV-S Experiment), using Adjusted SS for Tests

| Source | DF | Seq SS | Adj SS | Adj MS | F | P |
|---|---|---|---|---|---|---|
| Treatment | 2 | 0.748 | 0.748 | 0.374 | 42.80 | 0.000 |
| Replicates(Treatment) | 6 | 0.079 | 0.079 | 0.013 | 1.52 | 0.229 |
| Error | 18 | 0.157 | 0.157 | 0.009 | | |
| Total | 26 | 0.984 | | | | |

Fully Nested Analysis of Variance for concentration of MAA's in *A. cervicornis* (UV-S Experiment), using Adjusted SS for Tests

| Source | DF | Seq SS | Adj SS | Adj MS | F | P |
|---|---|---|---|---|---|---|
| Treatment | 5 | 2134222 | 2080680 | 416136 | 210.10 | 0.000 |
| Replicates(Treatment) | 12 | 6272 | 6272 | 523 | 0.26 | 0.992 |
| Error | 42 | 83188 | 83188 | 1981 | | |
| Total | 59 | 2223682 | | | | |

Fully Nested Analysis of Variance for concentration of MAA's in *P. furcata* (UV-S Experiment), using Adjusted SS for Tests

| Source | DF | Seq SS | Adj SS | Adj MS | F | P |
|---|---|---|---|---|---|---|
| Treatment | 2 | 524050 | 524050 | 262025 | 263.19 | 0.000 |
| Replicates(Treatment) | 6 | 423 | 423 | 70 | 0.07 | 0.998 |
| Error | 18 | 17920 | 17920 | 996 | | |
| Total | 26 | 542393 | | | | |

Fully Nested Analysis of Variance for concentration of photosynthetic pigments per coral tissue area in *A. cervicornis* (UV-S Experiment), using Adjusted SS for Tests

| Source | DF | Seq SS | Adj SS | Adj MS | F | P |
|---|---|---|---|---|---|---|
| Treatment | 5 | 669.31 | 634.26 | 126.85 | 72.99 | 0.000 |
| Replicates(Treatment) | 12 | 37.39 | 37.39 | 3.12 | 1.79 | 0.081 |
| Error | 42 | 72.99 | 72.99 | 1.738 | | |
| Total | 59 | 779.69 | | | | |

Fully Nested Analysis of Variance for concentration of photosynthetic pigments per zooxanthellae cell in *A. cervicornis* (UV-S Experiment), using Adjusted SS for Tests

| Source | DF | Seq SS | Adj SS | Adj MS | F | P |
|---|---|---|---|---|---|---|
| Treatment | 5 | 2018.91 | 2043.41 | 408.68 | 28.13 | 0.000 |
| Replicates(Treatment) | 12 | 155.37 | 155.37 | 12.95 | 0.89 | 0.562 |
| Error | 42 | 610.17 | 610.17 | 14.53 | | |
| Total | 59 | 2784.45 | | | | |

Fully Nested Analysis of Variance for concentration of photosynthetic pigments per coral tissue area in *P. furcata* (UV-S Experiment), using Adjusted SS for Tests

| Source | DF | Seq SS | Adj SS | Adj MS | F | P |
|---|---|---|---|---|---|---|
| Treatment | 2 | 1655.48 | 1655.48 | 827.74 | 201.33 | 0.000 |
| Replicates(Treatment) | 6 | 39.49 | 39.49 | 6.58 | 1.60 | 0.204 |
| Error | 18 | 74.00 | 74.00 | 4.11 | | |
| Total | 26 | 1768.97 | | | | |

Fully Nested Analysis of Variance for concentration of photosynthetic pigments per zooxanthellae cell in *P. furcata* (UV-S Experiment), using Adjusted SS for Tests

| Source | DF | Seq SS | Adj SS | Adj MS | F | P |
|---|---|---|---|---|---|---|
| Treatment | 2 | 7000.97 | 7000.97 | 3500.49 | 53.49 | 0.000 |
| Replicates(Treatment) | 6 | 180.85 | 180.85 | 30.14 | 0.46 | 0.828 |
| Error | 18 | 1177.85 | 1177.85 | 65.44 | | |
| Total | 26 | 8359.67 | | | | |

Fully Nested Analysis of Variance for zooxanthellae density in *A. cervicornis* (UV-S Experiment), using Adjusted SS for Tests

| Source | DF | Seq SS | Adj SS | Adj MS | F | P |
|---|---|---|---|---|---|---|
| Treatment | 5 | 0.380 | 0.345 | 0.069 | 1.65 | 0.168 |
| Replicates(Treatment) | 12 | 0.162 | 0.162 | 0.013 | 0.32 | 0.981 |
| Error | 42 | 1.758 | 1.758 | 0.042 | | |
| Total | 59 | 2.300 | | | | |

Fully Nested Analysis of Variance for the Mitotic Index of zooxanthellae in *A. cervicornis* (UV-S Experiment), using Adjusted SS for Tests

| Source | DF | Seq SS | Adj SS | Adj MS | F | P |
|---|---|---|---|---|---|---|
| Treatment | 5 | 0.0034 | 0.0040 | 0.0008 | 1.63 | 0.173 |
| Replicates(Treatment) | 12 | 0.0084 | 0.0084 | 0.0007 | 1.42 | 0.194 |
| Error | 42 | 0.0208 | 0.0208 | 0.0005 | | |
| Total | 59 | 0.0326 | | | | |

Fully Nested Analysis of Variance for zooxanthellae density in *P. furcata* (UV-S Experiment), using Adjusted SS for Tests

| Source | DF | Seq SS | Adj SS | Adj MS | F | P |
|---|---|---|---|---|---|---|
| Treatment | 2 | 0.345 | 0.345 | 0.173 | 3.14 | 0.068 |
| Replicates(Treatment) | 6 | 0.236 | 0.236 | 0.039 | 0.72 | 0.641 |
| Error | 18 | 0.989 | 0.989 | 0.055 | | |
| Total | 26 | 1.571 | | | | |

Fully Nested Analysis of Variance for the Mitotic Index of zooxanthellae in *P. furcata* (UV-S Experiment), using Adjusted SS for Tests

| Source | DF | Seq SS | Adj SS | Adj MS | F | P |
|---|---|---|---|---|---|---|
| Treatment | 2 | 0.0003 | 0.0003 | 0.00016 | 1.05 | 0.371 |
| Replicates(Treatment) | 6 | 0.0018 | 0.0018 | 0.00030 | 1.98 | 0.123 |
| Error | 18 | 0.0027 | 0.0027 | 0.00016 | | |
| Total | 26 | 0.0048 | | | | |

# APPENDIX II

Table 3.1. Concentration of the different photosynthetic pigments (chlorophylls, xanthophylls, and carotenoids) found in *A. cervicornis* during the UV-E Experiment. Numbers represent normalized concentration per coral tissue area (μg $cm^{-2}$) ± 1SD. Abbreviations: chlorophyll *a* (Chl *a*).

| | **Control** | | | **Hyzod** | | | **Saran** | | |
|---|---|---|---|---|---|---|---|---|---|
| **Pigment** | Apr 6 | Apr 28 | July 3 | Apr 6 | Apr 28 | July 3 | Apr 6 | Apr 28 | July 3 |
| Chlorophyll *a* | 2.91±0.50 | 2.63±0.29 | 5.73±1.19 | 2.89±0.06 | 2.94±0.07 | 5.54±0.90 | 4.77±1.67 | 4.88±0.27 | 9.37±1.12 |
| Chlorophyll $c_2$ | 0.65±0.11 | 0.63±0.06 | 1.65±0.28 | 0.80±0.09 | 0.65±0.03 | 1.17±0.16 | 1.11±0.22 | 1.15±0.08 | 1.90±0.21 |
| Peridinin | 1.22±0.19 | 1.15±0.07 | 1.51±0.40 | 1.68±0.08 | 1.21±0.10 | 2.56±0.47 | 2.14±0.33 | 2.21±0.14 | 3.07±0.52 |
| P-468 | 0.11±0.02 | 0.03±0.01 | 0.23±0.04 | 0.24±0.05 | 0.05±0.01 | 0.18±0.04 | 0.13±0.02 | 0.13±0.01 | 0.12±0.03 |
| P-457 | 0.33±0.04 | 0.12±0.03 | 0.40±0.05 | 0.34±0.02 | 0.46±0.03 | 0.22±0.06 | 0.49±0.13 | 0.28±0.04 | 0.34±0.08 |
| Diadinoxanthin | 0.51±0.03 | 0.55±0.03 | 2.05±0.31 | 0.32±0.01 | 0.55±0.02 | 1.51±0.20 | 0.59±0.16 | 0.79±0.10 | 1.90±0.17 |
| Diatoxanthin | -- | -- | -- | -- | 0.01±0.00 | -- | -- | -- | -- |
| Dinoxanthin | -- | 0.01±0.00 | 0.24±0.08 | -- | 0.01±0.00 | -- | 0.03±0.00 | 0.02±0.00 | 0.19±0.05 |
| Diadinochrome I | 0.06±0.01 | 0.06±0.02 | 0.11±0.03 | 0.07±0.02 | 0.06±0.01 | 0.13±0.02 | 0.10±0.02 | 0.09±0.03 | 0.12±0.03 |
| Diadinochrome II | 0.09±0.01 | 0.10±0.01 | -- | 0.10±0.01 | 0.09±0.01 | -- | 0.15±0.02 | 0.11±0.01 | -- |
| Diadinochrome epimer | -- | 0.05±0.01 | -- | 0.04±0.01 | 0.02±0.00 | -- | -- | -- | -- |
| Zeaxanthin | -- | 0.02±0.00 | -- | -- | 0.02±0.00 | 0.18±0.03 | 0.08±0.01 | 0.02±0.00 | -- |
| Lutein | -- | -- | -- | -- | -- | -- | 0.23±0.03 | -- | -- |
| β, β-carotene | 0.12±0.02 | 0.11±0.02 | 0.20±0.05 | 0.08±0.01 | 0.12±0.01 | 0.19±0.04 | 0.08±0.01 | 0.20±0.02 | 0.39±0.06 |
| Chl *a* allomer | 0.04±0.01 | -- | -- | -- | 0.02±0.00 | 0.16±0.04 | 0.12±0.03 | 0.02±0.00 | 0.07±0.01 |
| Chl *a* epimer | 0.69±0.06 | 0.38±0.09 | 0.90±0.35 | 0.52±0.07 | 0.48±0.03 | 0.50±0.07 | 0.97±0.04 | 0.81±0.07 | 0.87±0.12 |
| Pheophytin *a* | -- | -- | -- | -- | 0.12±0.02 | -- | -- | 0.24±0.05 | 0.37±0.08 |
| 9-*cis*-neoxanthin | -- | -- | -- | -- | -- | -- | -- | 0.03±0.00 | -- |

Table 3.2. Concentration of the different photosynthetic pigments (chlorophylls, xanthophylls, and carotenoids) found in *A. cervicornis* during the UV-E Experiment. Numbers represent normalized concentration per zooxanthellae cell (pg cell$^{-1}$) ± 1SD. Abbreviations: chlorophyll *a* (Chl *a*).

| | **Control** | | | **Hyzod** | | | **Saran** | | |
|---|---|---|---|---|---|---|---|---|---|
| **Pigment** | Apr 6 | Apr 28 | July 3 | Apr 6 | Apr 28 | July 3 | Apr 6 | Apr 28 | July 3 |
| Chlorophyll *a* | 3.65±0.75 | 2.91±0.72 | 6.71±1.93 | 2.50±0.25 | 2.29±0.37 | 5.34±1.10 | 2.72±0.65 | 3.14±0.52 | 7.75±0.47 |
| Chlorophyll $c_2$ | 0.74±0.16 | 0.71±0.16 | 1.81±0.29 | 0.88±0.12 | 0.51±0.10 | 1.11±0.16 | 0.72±0.13 | 0.73±0.10 | 1.59±0.14 |
| Peridinin | 1.91±0.13 | 1.75±0.51 | 1.64±0.43 | 1.14±0.31 | 0.95±0.19 | 2.33±0.33 | 1.17±0.20 | 1.41±0.18 | 2.56±0.42 |
| P-468 | 0.13±0.01 | 0.02±0.00 | 0.30±0.05 | 0.24±0.06 | 0.03±0.01 | 0.22±0.05 | 0.06±0.01 | 0.08±0.02 | 0.09±0.02 |
| P-457 | 0.41±0.05 | 0.08±0.01 | 0.52±0.10 | 0.36±0.07 | 0.12±0.04 | 0.20±0.09 | 0.24±0.05 | 0.17±0.01 | 0.27±0.08 |
| Diadinoxanthin | 0.45±0.09 | 0.46±0.06 | 2.37±0.56 | 0.41±0.04 | 0.42±0.06 | 1.43±0.21 | 0.42±0.14 | 0.52±0.13 | 1.60±0.15 |
| Diatoxanthin | -- | -- | -- | -- | 0.01±0.00 | -- | -- | -- | -- |
| Dinoxanthin | -- | 0.01±0.00 | 0.31±0.10 | -- | 0.01±0.00 | -- | -- | 0.02±0.00 | 0.14±0.06 |
| Diadinochrome I | 0.08±0.02 | 0.04±0.00 | 0.09±0.03 | 0.08±0.01 | 0.05±0.01 | 0.13±0.04 | 0.04±0.01 | 0.06±0.01 | 0.10±0.04 |
| Diadinochrome II | 0.12±0.02 | 0.07±0.02 | -- | 0.11±0.01 | 0.07±0.01 | -- | 0.07±0.01 | 0.08±0.02 | -- |
| Diadinochrome epimer | -- | 0.03±0.00 | -- | 0.04±0.00 | 0.01±0.00 | -- | -- | -- | -- |
| Zeaxanthin | -- | 0.01±0.00 | -- | -- | 0.02±0.00 | 0.18±0.07 | 0.04±0.00 | 0.02±0.00 | -- |
| Lutein | -- | -- | -- | -- | -- | -- | 0.10±0.00 | -- | -- |
| β, β-carotene | 0.09±0.02 | 0.09±0.01 | 0.24±0.08 | 0.07±0.01 | 0.09±0.01 | 0.18±0.05 | 0.04±0.01 | 0.13±0.02 | 0.31±0.02 |
| Chl *a* allomer | 0.05±0.00 | -- | -- | -- | 0.02±0.00 | 0.16±0.05 | 0.05±0.01 | 0.02±0.00 | 0.09±0.01 |
| Chl *a* epimer | 0.85±0.09 | 0.25±0.06 | 1.08±0.23 | 0.54±0.11 | 0.37±0.06 | 0.50±0.20 | 0.43±0.13 | 0.52±0.15 | 0.71±0.09 |
| Pheophytin *a* | 0.17±0.05 | -- | -- | -- | 0.10±0.02 | -- | 0.18±0.04 | 0.17±0.09 | 0.15±0.03 |
| 9-*cis*-neoxanthin | -- | -- | -- | -- | -- | -- | -- | 0.02±0.00 | -- |

Table 3.3. Concentration of the different photosynthetic pigments (chlorophylls, xanthophylls, and carotenoids) found in *P. furcata* during the UV-E Experiment. Numbers represent normalized concentration per coral tissue area (μg $cm^{-2}$) ± 1SD. Abbreviations: chlorophyll *a* (Chl *a*).

| | **Control** | | | **Hyzod** | | | **Saran** | | |
|---|---|---|---|---|---|---|---|---|---|
| **Pigment** | Apr 6 | Apr 28 | July 3 | Apr 6 | Apr 28 | July 3 | Apr 6 | Apr 28 | July 3 |
| Chlorophyll *a* | 8.16±1.01 | 9.19±0.35 | 8.85±0.72 | 9.58±0.76 | 11.26±0.78 | 21.77±3.42 | 8.65±1.47 | 19.63±4.36 | 34.07±2.46 |
| Chlorophyll $c_2$ | 1.99±0.24 | 1.99±0.12 | 2.57±0.22 | 2.70±0.32 | 2.57±0.16 | 5.32±0.69 | 2.87±0.39 | 4.04±0.76 | 8.91±0.59 |
| Peridinin | 5.31±0.40 | 4.49±1.06 | 3.77±0.60 | 5.66±0.20 | 4.52±0.14 | 12.28±0.87 | 5.54±0.83 | 7.73±1.57 | 20.07±1.31 |
| P-468 | 0.28±0.05 | 0.18±0.03 | 0.18±0.03 | 0.24±0.04 | 0.31±0.01 | 0.53±0.15 | 0.35±0.04 | 0.33±0.05 | 0.61±0.22 |
| P-457 | 0.66±0.07 | 0.36±0.09 | 0.31±0.11 | 0.92±0.05 | 0.68±0.10 | 1.23±0.17 | 0.87±0.10 | 1.13±0.17 | 2.12±0.17 |
| Diadinoxanthin | 1.07±0.02 | 1.11±0.04 | 2.32±0.24 | 1.18±0.15 | 0.80±0.12 | 4.25±0.90 | 1.00±0.13 | 1.39±0.27 | 5.72±0.38 |
| Diatoxanthin | 0.12±0.02 | 0.21±0.02 | -- | 0.29±0.06 | 0.20±0.03 | -- | 0.20±0.04 | 0.43±0.14 | -- |
| Neochrome | -- | -- | -- | -- | 0.09±0.02 | -- | 0.01±0.00 | 0.04±0.01 | -- |
| Diadinochrome I | 0.22±0.03 | 0.20±0.03 | 0.15±0.02 | 0.25±0.03 | 0.24±0.01 | 0.17±0.04 | 0.23±0.03 | 0.32±0.07 | 0.32±0.05 |
| Diadinochrome II | 0.26±0.03 | 0.24±0.05 | 0.15±0.03 | 0.30±0.02 | 0.30±0.02 | 0.19±0.04 | 0.28±0.05 | 0.39±0.06 | 0.34±0.03 |
| Diadinochrome epimer | 0.05±0.00 | 0.04±0.00 | -- | 0.07±0.01 | 0.08±0.01 | -- | 0.07±0.01 | 0.21±0.10 | -- |
| Zeaxanthin | -- | -- | 0.23±0.05 | 0.25±0.04 | -- | 0.42±0.11 | -- | 0.15±0.02 | 0.65±0.09 |
| Lutein | -- | -- | -- | 0.07±0.01 | -- | -- | -- | -- | -- |
| 19'-HF | -- | 0.04±0.00 | 0.20±0.01 | -- | 0.06±0.01 | 0.27±0.08 | -- | 0.10±0.03 | 0.12±0.02 |
| *cis*-19'-HF | -- | 0.07±0.01 | -- | -- | -- | -- | -- | 0.16±0.04 | 0.08±0.02 |
| β, β-carotene | 0.18±0.04 | 0.26±0.07 | 0.22±0.02 | 0.20±0.03 | 0.38±0.03 | 0.53±0.12 | 0.21±0.03 | 0.65±0.14 | 0.85±0.11 |
| Chl *a* epimer | 0.48±0.06 | 0.10±0.03 | 0.18±0.03 | 0.62±0.11 | 1.20±0.05 | 0.51±0.12 | 0.69±0.12 | 2.09±0.12 | 0.66±0.10 |
| Pheophytin *a* | 0.08±0.01 | 0.17±0.03 | -- | 0.56±0.15 | 0.55±0.07 | 0.51±0.24 | 0.50±0.09 | 1.29±0.24 | 1.69±0.33 |
| 9-*cis*-N | -- | -- | -- | 0.11±0.01 | -- | -- | 0.10±0.01 | -- | -- |
| Fucoxanthin | -- | -- | -- | -- | -- | -- | -- | 0.43±0.03 | 0.81±0.12 |
| Siphonein | -- | -- | -- | -- | -- | -- | -- | 0.22±0.08 | -- |

Table 3.4. Concentration of the different photosynthetic pigments (chlorophylls, xanthophylls, and carotenoids) found in *P. furcata* during the UV-E Experiment. Numbers represent normalized concentration per zooxanthellae cell (pg cell$^{-1}$) ± 1SD. Abbreviations: chlorophyll *a* (Chl *a*).

| | **Control** | | | **Hyzod** | | | **Saran** | | |
|---|---|---|---|---|---|---|---|---|---|
| **Pigment** | Apr 6 | Apr 28 | July 3 | Apr 6 | Apr 28 | July 3 | Apr 6 | Apr 28 | July 3 |
| Chlorophyll *a* | 10.07±2.12 | 10.84±0.62 | 23.02±3.67 | 13.39±2.51 | 18.41±3.43 | 36.90±4.55 | 11.04±1.50 | 23.19±4.70 | 63.84±9.79 |
| Chlorophyll $c_2$ | 1.65±0.24 | 2.36±0.21 | 6.64±1.02 | 3.16±0.70 | 4.18±0.75 | 9.21±1.37 | 3.66±0.28 | 4.79±0.84 | 16.68±2.49 |
| Peridinin | 4.22±0.25 | 5.26±1.37 | 9.68±1.77 | 6.51±0.93 | 7.32±1.10 | 21.15±3.54 | 7.05±0.58 | 9.14±1.67 | 37.40±5.19 |
| P-468 | 0.24±0.03 | 0.20±0.04 | 0.52±0.12 | 0.30±0.08 | 0.50±0.11 | 0.85±0.23 | 0.44±0.08 | 0.40±0.11 | 1.01±0.30 |
| P-457 | 0.57±0.05 | 0.40±0.09 | 0.80±0.26 | 1.06±0.12 | 1.12±0.24 | 2.11±0.29 | 1.10±0.18 | 1.34±0.27 | 4.03±0.72 |
| Diadinoxanthin | 0.96±0.03 | 1.07±0.16 | 5.73±0.95 | 1.38±0.31 | 1.33±0.35 | 7.29±1.69 | 1.28±0.10 | 1.65±0.28 | 10.69±1.57 |
| Diatoxanthin | 0.10±0.02 | 0.24±0.03 | -- | 0.28±0.06 | 0.32±0.06 | -- | 0.26±0.05 | 0.50±0.12 | -- |
| Neochrome | -- | -- | -- | -- | 0.13±0.01 | -- | 0.02±0.00 | 0.06±0.01 | -- |
| Diadinochrome I | 0.19±0.04 | 0.22±0.04 | 0.39±0.11 | 0.29±0.10 | 0.38±0.09 | 0.29±0.05 | 0.30±0.02 | 0.38±0.09 | 0.57±0.06 |
| Diadinochrome II | 0.23±0.04 | 0.26±0.02 | 0.39±0.10 | 0.36±0.06 | 0.47±0.08 | 0.33±0.06 | 0.35±0.03 | 0.47±0.13 | 0.63±0.09 |
| Diadinochrome epimer | 0.04±0.00 | 0.05±0.00 | -- | 0.09±0.02 | 0.12±0.03 | -- | 0.08±0.01 | 0.25±0.15 | -- |
| Zeaxanthin | -- | -- | 0.61±0.04 | 0.32±0.05 | -- | 0.69±0.15 | -- | 0.18±0.04 | 1.26±0.41 |
| Lutein | -- | -- | -- | 0.09±0.01 | -- | -- | -- | -- | -- |
| 19'-HF | -- | 0.04±0.00 | 0.45±0.11 | -- | 0.11±0.01 | 0.47±0.09 | -- | 0.11±0.03 | 0.29±0.02 |
| *cis*-19'-HF | -- | 0.07±0.01 | -- | -- | -- | -- | -- | 0.17±0.03 | 0.19±0.03 |
| β, β-carotene | 0.15±0.03 | 0.30±0.07 | 0.63±0.10 | 0.23±0.02 | 0.62±0.13 | 0.88±0.12 | 0.27±0.04 | 0.77±0.16 | 1.61±0.36 |
| Chl *a* epimer | 0.42±0.06 | 0.11±0.02 | 0.46±0.13 | 0.72±0.15 | 1.97±0.34 | 0.88±0.20 | 0.88±0.09 | 2.48±0.37 | 1.24±0.25 |
| Pheophytin *a* | 0.07±0.01 | 0.19±0.03 | -- | 0.61±0.11 | 0.93±0.21 | 0.77±0.32 | 0.65±0.07 | 1.51±0.45 | 1.10±0.18 |
| 9-*cis*-N | -- | -- | -- | 0.14±0.03 | -- | -- | 0.10±0.01 | -- | -- |
| Fucoxanthin | -- | -- | -- | -- | -- | -- | -- | 0.48±0.10 | 2.10±0.21 |
| Siphonein | -- | -- | -- | -- | -- | -- | -- | 0.25±0.07 | -- |

Table 3.5. Concentration of the different photosynthetic pigments (chlorophylls, xanthophylls, and carotenoids) found in *A. cervicornis* during the UV-S Experiment. Numbers represent normalized concentration per coral tissue area (μg $cm^{-2}$) ± 1SD. Abbreviations: chlorophyll *a* (Chl *a*).

| | **San Cristóbal** | | | **Old Buoy** | | | **El Palo** |
|---|---|---|---|---|---|---|---|
| **Pigment** | March 20 | Apr 29 | July 31 | March 20 | Apr 29 | July 31 | March 20 |
| Chlorophyll *a* | 2.25±0.27 | 3.99±0.35 | 4.21±0.56 | 3.99±0.36 | 3.61±0.08 | 5.91±0.23 | 3.05±0.29 |
| Chlorophyll $c_2$ | 0.50±0.04 | 1.04±0.03 | 0.77±0.07 | 0.80±0.22 | 1.06±0.03 | 1.21±0.09 | 0.70±0.12 |
| Peridinin | 0.82±0.22 | 2.38±0.27 | 1.97±0.32 | 2.26±0.42 | 3.12±0.06 | 4.08±0.24 | 1.81±0.26 |
| P-468 | -- | -- | -- | -- | 0.26±0.05 | -- | -- |
| P-457 | 0.16±0.01 | 0.29±0.06 | 0.18±0.05 | 0.28±0.06 | 0.34±0.05 | 0.50±0.03 | 0.23±0.07 |
| Diadinoxanthin | 0.75±0.13 | 1.14±0.04 | 1.30±0.17 | 0.66±0.07 | 0.63±0.05 | 0.95±0.05 | 0.53±0.08 |
| Diatoxanthin | 0.17±0.03 | 0.62±0.09 | 0.05±0.01 | 0.09±0.03 | 0.10±0.02 | 0.05±0.01 | 0.05±0.01 |
| Dinoxanthin | 0.10±0.04 | 0.18±0.04 | 0.09±0.02 | 0.13±0.04 | 0.14±0.03 | 0.15±0.02 | 0.08±0.02 |
| Diadinochrome I | -- | -- | 0.11±0.02 | -- | -- | 0.09±0.02 | -- |
| Diadinochrome II | -- | -- | -- | -- | -- | -- | -- |
| Diadinochrome epimer | -- | -- | -- | -- | -- | -- | -- |
| Zeaxanthin | -- | -- | -- | -- | -- | -- | -- |
| Lutein | -- | -- | -- | -- | -- | -- | 0.07±0.01 |
| β, β-carotene | 0.11±0.01 | 0.20±0.02 | 0.26±0.03 | 0.09±0.02 | 0.12±0.01 | 0.28±0.01 | 0.10±0.02 |
| Chl *a* allomer | 0.14±0.03 | 0.06±0.01 | 0.01±0.00 | 0.19±0.04 | 0.14±0.03 | 0.05±0.01 | 0.15±0.02 |
| Chl *a* epimer | 0.41±0.05 | 0.84±0.10 | 0.54±0.07 | 0.56±0.08 | 0.71±0.10 | 0.65±0.09 | 0.42±0.04 |
| Pheophytin *a* | -- | -- | -- | -- | 0.09±0.03 | -- | -- |
| 9-*cis*-neoxanthin | -- | -- | -- | -- | -- | -- | -- |
| Fucoxanthin | -- | 0.11±0.02 | 0.13±0.03 | -- | 0.05±0.00 | 0.12±0.02 | -- |

Table 3.6. Concentration of the different photosynthetic pigments (chlorophylls, xanthophylls, and carotenoids) found in *A. cervicornis* during the UV-S Experiment. Numbers represent normalized concentration per zooxanthellae cell (pg cell$^{-1}$) ± 1SD. Abbreviations: chlorophyll *a* (Chl *a*).

| | **San Cristóbal** | | | **Old Buoy** | | | **El Palo** |
|---|---|---|---|---|---|---|---|
| **Pigment** | March 20 | Apr 29 | July 31 | March 20 | Apr 29 | July 31 | March 20 |
| Chlorophyll *a* | 1.61±0.32 | 2.73±0.16 | 3.97±0.45 | 3.52±0.50 | 6.09±1.48 | 7.73±1.75 | 3.05±0.29 |
| Chlorophyll $c_2$ | 0.37±0.07 | 0.61±0.08 | 0.74±0.11 | 0.69±0.18 | 2.17±0.05 | 1.64±0.49 | 0.42±0.10 |
| Peridinin | 0.56±0.16 | 1.68±0.10 | 1.86±0.27 | 1.98±0.43 | 5.35±0.96 | 5.30±1.13 | 1.10±0.27 |
| P-468 | -- | -- | -- | -- | 0.57±0.12 | -- | -- |
| P-457 | 0.12±0.02 | 0.19±0.06 | 0.17±0.05 | 0.24±0.07 | 0.75±0.10 | 0.66±0.15 | 0.14±0.05 |
| Diadinoxanthin | 0.54±0.13 | 0.76±0.03 | 1.21±0.07 | 0.58±0.10 | 1.06±0.12 | 1.24±0.27 | 0.34±0.13 |
| Diatoxanthin | 0.12±0.03 | 0.41±0.10 | 0.05±0.01 | 0.05±0.02 | 0.23±0.03 | 0.06±0.01 | 0.03±0.01 |
| Dinoxanthin | 0.07±0.02 | 0.12±0.04 | 0.09±0.02 | 0.08±0.01 | 0.30±0.05 | 0.20±0.04 | 0.05±0.01 |
| Diadinochrome I | -- | -- | 0.10±0.01 | -- | -- | 0.12±0.02 | -- |
| Diadinochrome II | -- | -- | -- | -- | -- | -- | -- |
| Diadinochrome epimer | -- | -- | -- | -- | -- | -- | -- |
| Zeaxanthin | -- | -- | -- | -- | -- | -- | -- |
| Lutein | -- | -- | -- | -- | -- | -- | 0.02±0.00 |
| β, β-carotene | 0.08±0.01 | 0.15±0.01 | 0.25±0.02 | 0.08±0.01 | 0.22±0.06 | 0.37±0.09 | 0.06±0.01 |
| Chl *a* allomer | 0.10±0.03 | 0.04±0.01 | 0.01±0.00 | 0.16±0.03 | 0.31±0.05 | 0.07±0.01 | 0.09±0.02 |
| Chl *a* epimer | 0.29±0.05 | 0.56±0.08 | 0.52±0.09 | 0.50±0.04 | 1.56±0.13 | 0.85±0.12 | 0.25±0.04 |
| Pheophytin *a* | -- | -- | -- | -- | | -- | -- |
| 9-*cis*-neoxanthin | -- | -- | -- | -- | -- | -- | -- |
| Fucoxanthin | -- | 0.07±0.02 | 0.12±0.02 | -- | 0.11±0.02 | 0.15±0.03 | -- |

Table 3.7. Concentration of the different photosynthetic pigments (chlorophylls, xanthophylls, and carotenoids) found in transplanted colonies of *A. cervicornis* during the UV-S Experiment. Numbers represent normalized concentration per coral tissue area (µg $cm^{-2}$) ± 1SD. Abbreviations: chlorophyll *a* (Chl *a*).

| | **EP-SC** | | **OB-SC** | | **SC-OB** | | **EP-OB** | |
|---|---|---|---|---|---|---|---|---|
| **Pigment** | Apr 29 | July 31 | Apr 29 | July 31 | Apr 29 | July 31 | Apr 29 | July 31 |
| Chlorophyll *a* | 3.62±0.44 | 3.74±0.79 | 1.22±0.02 | 1.87±0.44 | 3.55±0.04 | 3.80±0.23 | 3.71±0.20 | 5.21±0.31 |
| Chlorophyll $c_2$ | 1.35±0.10 | 0.79±0.18 | 0.54±0.04 | 0.28±0.05 | 0.99±0.14 | 0.71±0.05 | 1.01±0.08 | 1.00±0.05 |
| Peridinin | 1.35±0.10 | 1.96±0.32 | 1.12±0.08 | 0.88±0.07 | 2.28±0.20 | 1.73±0.13 | 3.55±0.10 | 3.66±0.20 |
| P-468 | 0.26±0.03 | 0.10±0.02 | 0.03±0.00 | -- | 0.16±0.04 | -- | 0.23±0.03 | -- |
| P-457 | 0.31±0.04 | 0.20±0.06 | 0.12±0.03 | 0.12±0.02 | 0.27±0.05 | 0.21±0.01 | 0.32±0.05 | 0.41±0.02 |
| Diadinoxanthin | 0.57±0.05 | 0.71±0.16 | 0.18±0.01 | 0.29±0.09 | 0.78±0.08 | 0.72±0.03 | 0.44±0.03 | 0.71±0.03 |
| Diatoxanthin | 0.56±0.06 | 0.04±0.01 | 0.14±0.05 | 0.03±0.01 | 0.38±0.10 | 0.03±0.00 | 0.29±0.07 | 0.04±0.01 |
| Dinoxanthin | 0.20±0.03 | 0.08±0.02 | 0.06±0.01 | 0.05±0.01 | 0.13±0.03 | 0.08±0.01 | 0.15±0.04 | 0.09±0.02 |
| Diadinochrome I | -- | 0.05±0.01 | -- | 0.06±0.01 | -- | 0.05±0.00 | -- | 0.11±0.04 |
| Diadinochrome II | -- | -- | -- | -- | -- | -- | -- | -- |
| Diadinochrome epimer | -- | -- | -- | -- | -- | -- | -- | -- |
| Zeaxanthin | -- | -- | -- | -- | -- | -- | -- | -- |
| Lutein | -- | -- | -- | -- | -- | -- | -- | -- |
| β, β-carotene | 0.19±0.01 | 0.16±0.03 | 0.05±0.02 | 0.07±0.02 | 0.14±0.01 | 0.23±0.01 | 0.14±0.01 | 0.25±0.03 |
| Chl *a* allomer | 0.07±0.01 | 0.02±0.00 | 0.05±0.01 | 0.02±0.00 | 0.32±0.05 | 0.03±0.01 | 0.14±0.03 | 0.04±0.01 |
| Chl *a* epimer | 0.78±0.10 | 0.44±0.10 | 0.23±0.08 | 0.24±0.05 | 0.69±0.07 | 0.45±0.03 | 0.63±0.18 | 0.60±0.15 |
| Pheophytin *a* | 0.08±0.01 | -- | -- | -- | -- | -- | 0.06±0.01 | -- |
| 9-*cis*-neoxanthin | -- | -- | -- | -- | -- | -- | -- | -- |
| Fucoxanthin | 0.06±0.01 | 0.06±0.01 | -- | 0.03±0.01 | 0.07±0.01 | 0.07±0.01 | 0.04±0.01 | 0.09±0.01 |

Table 3.8. Concentration of the different photosynthetic pigments (chlorophylls, xanthophylls, and carotenoids) found in transplanted colonies of *A. cervicornis* during the UV-S Experiment. Numbers represent normalized concentration per zooxanthellae cell (pg cell$^{-1}$) ± 1SD. Abbreviations: chlorophyll *a* (Chl *a*).

| | **EP-SC** | | **OB-SC** | | **SC-OB** | | **EP-OB** | |
|---|---|---|---|---|---|---|---|---|
| **Pigment** | Apr 29 | July 31 | Apr 29 | July 31 | Apr 29 | July 31 | Apr 29 | July 31 |
| Chlorophyll *a* | 2.62±0.26 | 4.75±1.63 | 2.75±0.18 | 2.62±0.88 | 1.91±0.06 | 5.82±1.21 | 5.07±0.05 | 9.71±1.92 |
| Chlorophyll $c_2$ | 0.62±0.01 | 1.00±0.35 | 1.14±0.11 | 0.38±0.11 | 0.65±0.03 | 1.07±0.20 | 1.58±0.06 | 1.85±0.36 |
| Peridinin | 0.62±0.01 | 2.44±0.76 | 0.89±0.07 | 1.22±0.24 | 1.25±0.03 | 2.61±0.50 | 4.40±0.28 | 6.74±1.19 |
| P-468 | 0.14±0.02 | 0.14±0.03 | 0.07±0.00 | -- | 0.08±0.03 | -- | 0.34±0.04 | -- |
| P-457 | 0.16±0.03 | 0.26±0.08 | 0.25±0.04 | 0.16±0.03 | 0.13±0.05 | 0.32±0.06 | 0.49±0.06 | 0.77±0.18 |
| Diadinoxanthin | 0.37±0.02 | 0.91±0.30 | 0.37±0.01 | 0.39±0.16 | 0.39±0.04 | 1.08±0.18 | 0.41±0.01 | 1.35±0.29 |
| Diatoxanthin | 0.29±0.03 | 0.06±0.01 | 0.28±0.07 | 0.03±0.01 | 0.19±0.08 | 0.05±0.01 | 0.43±0.07 | 0.06±0.01 |
| Dinoxanthin | 0.11±0.01 | 0.10±0.02 | 0.11±0.02 | 0.08±0.01 | 0.06±0.02 | 0.11±0.02 | 0.23±0.04 | 0.17±0.04 |
| Diadinochrome I | -- | 0.06±0.01 | -- | 0.09±0.02 | -- | 0.08±0.01 | -- | 0.19±0.05 |
| Diadinochrome II | -- | -- | -- | -- | -- | -- | -- | -- |
| Diadinochrome epimer | -- | -- | -- | -- | -- | -- | -- | -- |
| Zeaxanthin | -- | -- | -- | -- | -- | -- | -- | -- |
| Lutein | -- | -- | -- | -- | -- | -- | -- | -- |
| β, β-carotene | 0.11±0.00 | 0.20±0.06 | 0.06±0.00 | 0.10±0.03 | 0.11±0.02 | 0.34±0.05 | 0.21±0.02 | 0.46±0.09 |
| Chl *a* allomer | 0.04±0.01 | 0.04±0.00 | 0.09±0.01 | 0.03±0.00 | 0.16±0.04 | 0.04±0.01 | 0.21±0.03 | 0.06±0.01 |
| Chl *a* epimer | 0.41±0.06 | 0.57±0.11 | 0.47±0.10 | 0.36±0.07 | 0.35±0.06 | 0.69±0.08 | 0.95±0.15 | 1.11±0.13 |
| Pheophytin *a* | 0.04±0.00 | -- | -- | -- | -- | -- | 0.09±0.01 | -- |
| 9-*cis*-neoxanthin | -- | -- | -- | -- | -- | -- | -- | -- |
| Fucoxanthin | 0.03±0.00 | 0.07±0.01 | -- | 0.05±0.01 | 0.03±0.00 | 0.10±0.02 | 0.06±0.01 | 0.16±0.03 |

Table 3.9. Concentration of the different photosynthetic pigments (chlorophylls, xanthophylls, and carotenoids) found in *P. furcata* during the UV-S Experiment. Numbers represent normalized concentration per coral tissue area (μg $cm^{-2}$) ± 1SD. Abbreviations: chlorophyll *a* (Chl *a*).

| | **Control** | | | **Hyzod** | | | **UV** | | |
|---|---|---|---|---|---|---|---|---|---|
| **Pigment** | March 20 | Apr 29 | July 31 | March 20 | Apr 29 | July 31 | March 20 | Apr 29 | July 31 |
| Chlorophyll *a* | 11.70±1.83 | 10.52±0.16 | 11.74±1.35 | 10.55±0.99 | 15.14±0.16 | 14.72±1.22 | 17.08±2.28 | 7.65±0.11 | 6.06±0.49 |
| Chlorophyll $c_2$ | 1.97±0.42 | 2.40±0.04 | 2.23±0.14 | 1.84±0.53 | 3.09±0.03 | 3.07±0.11 | 4.19±0.44 | 1.82±0.05 | 1.39±0.14 |
| Peridinin | 5.15±0.80 | 4.81±0.34 | 4.74±0.13 | 4.23±0.52 | 7.02±0.50 | 5.70±0.31 | 7.53±0.99 | 3.45±0.11 | 2.49±0.24 |
| P-468 | 0.15±0.04 | 0.26±0.06 | 0.08±0.01 | 0.15±0.06 | 0.41±0.02 | 0.38±0.03 | 0.40±0.07 | 0.22±0.01 | 0.17±0.03 |
| P-457 | 0.57±0.09 | 0.47±0.05 | 0.72±0.01 | 0.44±0.08 | 0.79±0.05 | 0.45±0.10 | 1.24±0.14 | 0.56±0.02 | 0.27±0.04 |
| Diadinoxanthin | 2.28±0.36 | 0.52±0.01 | 1.17±0.31 | 1.23±0.25 | 0.73±0.03 | 2.06±0.23 | 1.83±0.48 | 0.47±0.07 | 0.89±0.07 |
| Diatoxanthin | 0.24±0.05 | 1.05±0.13 | 0.25±0.06 | 0.23±0.05 | 0.74±0.11 | 0.57±0.12 | 0.47±0.12 | 0.24±0.02 | 0.25±0.03 |
| Dinoxanthin | 0.37±0.09 | 0.06±0.01 | 0.15±0.06 | 0.20±0.05 | 0.12±0.01 | 0.32±0.05 | 0.33±0.07 | 0.06±0.02 | 0.11±0.02 |
| Neochrome | -- | -- | -- | -- | -- | -- | 0.06±0.01 | -- | -- |
| Diadinochrome I | -- | 0.15±0.01 | 0.14±0.02 | 0.11±0.03 | 0.15±0.01 | 0.13±0.02 | 0.22±0.06 | 0.12±0.04 | 0.09±0.02 |
| Diadinochrome II | -- | 0.29±0.03 | 0.21±0.07 | 0.12±0.02 | 0.31±0.02 | 0.28±0.05 | 0.37±0.13 | 0.24±0.09 | 0.18±0.04 |
| Diadinochrome epimer | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| Zeaxanthin | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| β, β-carotene | 0.39±0.09 | 0.36±0.01 | 0.37±0.03 | 0.22±0.05 | 0.30±0.02 | 0.28±0.09 | 0.47±0.07 | 0.20±0.01 | 0.19±0.01 |
| Chl *a* epimer | 0.19±0.04 | 0.27±0.02 | 0.35±0.05 | 0.23±0.07 | 0.52±0.02 | 0.12±0.02 | 0.33±0.09 | 0.28±0.02 | 0.02±0.01 |
| Pheophytin *a* | -- | -- | -- | -- | -- | -- | -- | -- | 0.08±0.02 |
| 9-*cis*-neoxanthin | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| Fucoxanthin | -- | 0.04±0.00 | 0.14±0.02 | 0.04±0.00 | 0.07±0.01 | 0.20±0.07 | 0.21±0.05 | 0.06±0.01 | 0.10±0.02 |
| Siphonein | -- | 0.41±0.05 | 0.12±0.01 | -- | 0.29±0.12 | 0.44±0.07 | -- | 0.08±0.01 | 0.13±0.03 |

Table 3.10. Concentration of the different photosynthetic pigments (chlorophylls, xanthophylls, and carotenoids) found in *P. furcata* during the UV-S Experiment. Numbers represent normalized concentration per zooxanthellae cell (pg cell$^{-1}$) ± 1SD. Abbreviations: chlorophyll *a* (Chl *a*).

| | **Control** | | | **Hyzod** | | | **UV** | | |
|---|---|---|---|---|---|---|---|---|---|
| **Pigment** | March 20 | Apr 29 | July 31 | March 20 | Apr 29 | July 31 | March 20 | Apr 29 | July 31 |
| Chlorophyll *a* | 9.62±2.42 | 12.54±1.92 | 28.60±3.97 | 14.70±4.87 | 26.04±6.97 | 14.98±4.12 | 14.03±4.84 | 9.79±0.94 | 6.51±0.76 |
| Chlorophyll $c_2$ | 2.08±1.02 | 2.86±0.44 | 5.43±0.49 | 2.95±1.37 | 5.25±1.30 | 3.03±0.68 | 3.55±1.30 | 2.32±0.16 | 1.49±0.18 |
| Peridinin | 5.32±2.07 | 5.83±1.20 | 11.48±0.60 | 5.92±2.02 | 12.46±3.96 | 5.76±1.48 | 6.37±2.40 | 4.38±0.29 | 2.66±0.30 |
| P-468 | 0.18±0.03 | 0.32±0.07 | 0.20±0.01 | 0.18±0.09 | 0.68±0.21 | 0.38±0.06 | 0.38±0.07 | 0.28±0.01 | 0.18±0.04 |
| P-457 | 0.59±0.17 | 0.57±0.11 | 1.75±0.08 | 0.63±0.12 | 1.39±0.50 | 0.50±0.12 | 1.03±0.14 | 0.51±0.20 | 0.29±0.07 |
| Diadinoxanthin | 2.39±0.85 | 0.62±0.07 | 2.87±0.83 | 1.64±0.54 | 1.28±0.38 | 2.07±0.61 | 1.33±0.27 | 0.62±0.14 | 0.96±0.11 |
| Diatoxanthin | 0.31±0.06 | 1.26±0.19 | 0.61±0.15 | 0.21±0.07 | 1.77±0.25 | 0.62±0.25 | 0.30±0.08 | 0.30±0.01 | 0.27±0.03 |
| Dinoxanthin | 0.41±0.04 | 0.08±0.01 | 0.41±0.09 | 0.27±0.10 | 0.20±0.04 | 0.30±0.07 | 0.23±0.04 | 0.09±0.01 | 0.11±0.02 |
| Neochrome | -- | -- | -- | -- | -- | -- | 0.05±0.00 | -- | -- |
| Diadinochrome I | -- | 0.17±0.03 | 0.33±0.05 | 0.12±0.05 | 0.26±0.05 | 0.13±0.05 | 0.17±0.04 | 0.14±0.02 | 0.10±0.03 |
| Diadinochrome II | -- | 0.36±0.08 | 0.50±0.16 | 0.17±0.07 | 0.53±0.12 | 0.29±0.05 | 0.29±0.08 | 0.29±0.03 | 0.19±0.04 |
| Diadinochrome epimer | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| Zeaxanthin | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| β, β-carotene | 0.40±0.13 | 0.44±0.08 | 0.90±0.06 | 0.28±0.11 | 0.53±0.17 | 0.32±0.13 | 0.40±0.15 | 0.25±0.02 | 0.19±0.01 |
| Chl *a* epimer | 0.30±0.11 | 0.32±0.06 | 0.83±0.20 | 0.28±0.07 | 0.88±0.07 | 0.12±0.02 | 0.28±0.05 | 0.35±0.02 | 0.02±0.01 |
| Pheophytin *a* | -- | -- | -- | -- | -- | -- | -- | -- | 0.13±0.02 |
| 9-*cis*-neoxanthin | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| Fucoxanthin | -- | 0.05±0.01 | 0.35±0.05 | 0.07±0.02 | 0.12±0.02 | 0.20±0.03 | 0.14±0.06 | 0.07±0.01 | 0.09±0.02 |
| Siphonein | -- | 0.51±0.15 | 0.28±0.02 | -- | 0.60±0.22 | 0.47±0.07 | -- | 0.10±0.02 | 0.14±0.03 |